IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Inventors:	**Buckey et al.**
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Examiner:	**San Ming Hui**
Customer No.:	**26259**
Group Art Unit:	**1617**
Confirmation No.:	**1958**
Title:	**Methods of Preventing or Treating Motion Sickness**

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I hereby certify that this paper is being electronically submitted on the date indicated above to the Commissioner for Patents, U.S. Patent & Trademark Office (AF).

By _____
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Commissioner for Patents
U.S. Patent & Trademark Office
(AF)

APPEAL BRIEF

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I. Real Party in Interest

The real party in interest is Trustees of Dartmouth College.

II. Related Appeals and Interferences

There are no related appeals or interferences.

III. Status of Claims

Claim 1 is pending in this application.

Claim 1 has been rejected and is on appeal. A claim appendix including the text of the appealed claim is attached.

IV. Status of Amendments

The claim amendments filed in November 21, 2006 were entered. The rejections to claim 1 were maintained in the Final Rejection dated July 14, 2008.

V. Summary of the Claimed Subject Matter

Claim 1 defines a method for decreasing the signs and symptoms of motion sickness that comprises administering to a subject an effective amount of a halogenated pheniramine to decrease the signs or symptoms of motion sickness, wherein the halogenated pheniramine consists of chlorpheniramine or enantiomers thereof, wherein the halogenated pheniramine is administered orally or topically, and wherein said halogenated pheniramine is administered at a dose of 12 mg. See page 5, lines 8-28, page 6, lines 1-15, and pages 5-6, Tables 1 and 2, where all aspects of the invention as claimed are explicitly taught, including the use of a dose of 12 mg chlorpheniramine.

VI. Grounds of Rejection to be Reviewed on Appeal

Whether claim 1 should stand rejected under 35 U.S.C. §103(a) as being unpatentable over Ueno *et al.* (1988), in view of *Drug Information Handbook*, the *British Medical Journal* (1970), Weinstein *et al.* (1997), and Kohl *et al.* (1991).

VII. Arguments

A. The Rejection of Claim 1 Under 35 U.S.C. §103(a) Over Ueno et al. (1988), in View of *Drug Information Handbook*, the *British Medical Journal* (1970), Weinstein et al. (1997), and Kohl et al. (1991) Should Be Withdrawn

The Examiner suggests that it would have been *prima facie* obvious for one of ordinary skill in the art to employ a 12 mg dose of chlorpheniramine in a method of treating motion sickness since Ueno *et al.* (1988) teaches treatment of motion sickness with a 20 mg/kg dose in *S. murinus*, the *Drug Information Handbook* teaches that human dose of chlorpheniramine is 8-12 mg every 8-12 hours, the *British Medical Journal* teaches antihistamines are useful for treating vomiting, a symptom of motion sickness, Weinstein *et al.* (1997) teach common agents for treating symptoms of motion sickness are antihistamines, and Kohl *et al.* (1991) teach that an oral antihistamine terfenadine can be used to treat motion sickness. The Examiner suggests that one of skill would have been motivated to employ chlorpheniramine at a 12 mg dose orally to treat motion sickness because the human dose of chlorpheniramine encompasses the dosage herein claimed, and that various known anti-histamines are administered orally when used to treat motion sickness (citing *British Medical Journal*, Weintein *et al.*, and Kohl *et al.*). Therefore, the Examiner suggests one of skill would administer chlorpheniramine, an anti-histamine, orally to treat motion sickness since chlorpheniramine is an old and well-known compound. Appellants respectfully disagree with the Examiner's analysis and conclusions regarding the cited references.

In rejecting claims under 35 U.S.C. §103, the Examiner bears the initial burden of presenting a *prima facie* case of obviousness. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443,

1444 (Fed. Cir. 1992). In order to meet that burden the Examiner must provide a reason, based on the prior art, or knowledge generally available in the art as to why it would have been obvious to one of ordinary skill in the art to arrive at the claimed invention. *Ashland Oil, Inc. v. Delta Resins & Refractories, Inc.*, 776 F.2d 281, 297, n.24, 227 USPQ 657, 667, n.24 (Fed. Cir. 1985). Further, a rejection based on section 103 must rest upon a factual basis rather than conjecture, or speculation. "Where the legal conclusion [of obviousness] is not supported by facts it cannot stand." *In re Warner*, 379 F.2d 1011, 1017, 154 USPQ 173, 178 (CCPA 1967). See also *In re Lee*, 277 F.3d 1338, 1344, 61 USPQ2d 1430, 1434 (Fed. Cir, 2002) and *In re Kahn*, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006). Appellants believe that the Examiner has not met the initial burden of establishing why the prior art relied on would have led one of ordinary skill in this art to arrive at the claimed invention, and has not provided any factual basis, only speculation or conjecture, for the position that the combined references demonstrate that a dose of 12 mg of chlorpheniramine in a human orally would decrease the signs or symptoms of motion sickness.

Ueno et al. (1988) disclose use of several different drugs, including chlorpheniramine, to treat symptoms of motion sickness in an animal model for motion sickness. The chlorpheniramine was administered subcutaneously at a dose of 20 mg/kg. Nowhere does this reference teach use of any other dose or route of administration of chlorpheniramine, especially not a dose that is 2 orders of magnitude lower than 20 mg/kg. It should be noted that a 12 mg dose in a human, as is claimed in claim 1, is equivalent to a dose of about 0.2 mg/kg (12 mg divided by average human body weight of 70 kg). Most importantly, it is a

well-established general principle of pharmacology, taught in basic textbooks (e.g., *Goodman & Gilman's: The Pharmacological Basis of Therapeutics*, 10th edition. 2001. J.G. Hardman and L.E. Limbird (eds.), McGraw Hill: New York) that a pharmacological effect of a drug, its efficacy, is defined by the principle of dose-response. In the case of a drug such as chlorpheniramine, a drug that produces its effects through activity on receptors (see chapter 25 of *Goodman & Gilman's: The Pharmacological Basis of Therapeutics*, 10th edition. 2001. J.G. Hardman and L.E. Limbird (eds.), McGraw Hill: New York, copy provided with response filed May 7, 2007; a copy is included in Evidence Appendix for your consideration), a dose-response relationship is defined routinely as an s-shaped curve (see page 39 of chapter 2 from *Goodman & Gilman's: The Pharmacological Basis of Therapeutics*, 10th edition. 2001. J.G. Hardman and L.E. Limbird (eds.), McGraw Hill: New York, copy provided with response filed May 7, 2007; a copy is included in Evidence Appendix for your consideration). With such a dose-response relationship, pharmacological activity, or drug efficacy, increases as dose increases, in a steady, almost linear manner at some doses. However, at very low doses, there is often no detectable activity. As a result, it is not supported by general principles of pharmacology that one of skill would understand or expect that a dose of a drug that is 2-orders of magnitude lower than a tested dose would produce a pharmacological effect or have efficacy.

Additionally, teaching of a dose given by an entirely different route of administration (subcutaneously by Ueno et al. versus orally as claimed) does not provide one of skill with en expectation of success of using a drug effectively by another route. It is a general principle of pharmacology that efficacy

of a drug is effected by the route of administration. In the case of the instant invention, the Examiner has suggested that data from subcutaneous administration of a drug predicts what one of skill would expect to see with an entirely different route. In the specification as filed, the data provided to enable the claimed invention is based on administration of chlorpheniramine orally to humans. In the study by Ueno et al. (1988) cited by the Examiner, chlorpheniramine was shown to have anti-nausea effects when administered subcutaneously at a much higher dose, not orally. It is also a general principle of pharmacokinetics (the scientific discipline that studies how a drug arrives at its site of action to produce its effects, and how a route of administration affects the required effective dose of a drug) that a drug administered subcutaneously would have a different dose-response relationship for efficacy than that same drug would have if administered orally (see chapter 1 of *Goodman & Gilman's: The Pharmacological Basis of Therapeutics*, 10th edition. 2001. J.G. Hardman and L.E. Limbird (eds.), McGraw Hill: New York, copy provided with response filed May 7, 2007; a copy is included in Evidence Appendix for your consideration). Therefore, contrary to the Examiner's suggestion, one of skill would not use data on subcutaneous administration of a much higher dose of a drug to predict a dose level that would be effective orally for the same drug at a much lower dose.

The Examiner suggests that the reference *Drug Information Handbook* teaches that a human oral dose of chlorpheniramine is 8-12 mg every 8-12 hours, and further that it is this reference that the Examiner relies on to establish a *prima facie* case of obviousness, not the reference of Ueno et al. (1988). However, there are several different statements under "usual dosage" of

chlorpheniramine in this reference. For example, oral doses in children are stated to start at 0.35 mg/kg/day for chlorpheniramine and for adults given chlorpheniramine i.m., i.v., or s.c., the maximum dosage stated is 20 mg. These statements indicate that the route of administration affects dose just as the indication being treated. In the *Drug Information Handbook* the doses listed refer to the approved indication for chlorpheniramine which is NOT motion sickness treatment but treatment of symptoms of an allergic reaction (anti-histamine activity). In order to determine what an effective dosage would be for motion sickness, one of skill in the art would need to see data on the drug in question, chlorpheniramine, for treatment of motion sickness. Moreover, in light of the fact that the paper of Ueno *et al.* (1988) disclose use of a much larger dose of chlorpheniramine to treat motion sickness in an animal, one of skill would not be motivated to try these lower doses of chlorpheniramine that have only be shown to be effective for anti-histamine activity NOT motion sickness.

The Examiner has then suggested that one of skill would turn to references on treatment of motion sickness that describe use of antihistamines for motion sickness or vomiting (*British Medical Journal* 1970; Weinstein *et al.* 1997; Kohl *et al.* 1991). However, if one carefully reviews each of these references it is seen that the dosages of the drugs discussed are <u>much higher</u> than 12 mg. Again, consistent with the teaching of Ueno *et al.* (1988) and NOT consistent with the teaching of the *Drug Information Handbook*. British Medical Journal (1970) teaches that the antihistamines cyclizine, meclazine, and promethazine are useful for treating the symptom of vomiting at doses of 25 to 50 mg when tablets are given orally or 1 mg/ml when an elixir

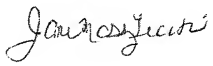
or syrup is taken orally (equivalent to from 60-70 mg based on human body weight). Nowhere does this reference mention chlorpheniramine nor any dose less than 25 mg orally. Weinstein et al. (1997) teaches use of over-the-counter antihistamines for motion sickness, specifically dimenhydrinate and cyclizine. The doses taught in this reference are 50 mg, doses much higher than 12 mg. Nowhere does this paper teach or suggest use of lower doses of any antihistamine to treat motion sickness. Additionally, a careful reading of the second column of pate 392 of the paper reveals that even though many antihistamines have been tested for their ability to prevent motion sickness "a minority of these drugs have indicated adequate therapeutic utility…". Therefore, this paper is actually providing one of skill with a reason to doubt that data on antihistamines as a class will predict the activity of any particular antihistamine. Kohl *et al.* (1991) disclose that terfenadine, an antihistamine that does not cross the blood brain barrier very effectively, at a dose of 300 mg orally can be used to control nausea induced by a rotating chair. No other dose of terfenadine is shown to be effective nor is there any teaching of a dose of chlorpheniramine. Each of the references that the Examiner relies on teaches either alone or when combined a dose of an entirely different antihistamine drug for treatment of motion sickness at a dose much larger than the dose claimed (12 mg). Moreover, without any data provided on the effect of chlorpheniramine to treat motion sickness at a dose level near the claimed dose (Ueno *et al.* teach a much higher dose of chlorpheniramine) it is not possible for one of skill to make or use the claimed invention.

In order to establish a *prima facie* case of obviousness, three basic criteria must be met. MPEP 2143. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art must teach or suggest all claim limitations. Clearly, the cited prior art fails to teach the invention as claimed which is use of chlorpheniramine at a specific dose to decrease the signs and symptoms of motion sickness. The art, when combined, teaches use of chlorpheniramine to treat symptoms of motion sickness only at much higher doses, 2 orders of magnitude higher doses, teaches chlorpheniramine to treat an allergic reaction, or teaches use of entirely different drugs to treat motion sickness but also at higher doses. One of skill would not expect that data on an entirely different drug at a much higher dose to suggest that chlorpheniramine given orally at 12 mg would be an effective drug to decrease the signs and symptoms of motion sickness. The Examiner's conclusions are without basis in the general principles and standards of pharmacology, and as a result are merely conjecture or speculation.

The presence or absence of a motivation to combine references is a question of fact, *In re Dembiczak*, 175 F.3d 994, 1000 (Fed. Cir. 1999), which is evaluated under the substantial evidence standard. *Gartside*, 203 F.3d at 1316. In the present case, the cited prior art simply provides no substantial evidence which supports the obviousness of decreasing the signs and symptoms of motion sickness by administering to a subject a 12 mg oral dose of chlorpheniramine. Thus, the cited prior art references cannot be held to substantiate this obviousness

rejection under 35 U.S.C. 103(a). Appellants therefore assert that Ueno *et al.* (1988), in view of *Drug Information Handbook*, the *British Medical Journal* (1970), Weinstein *et al.* (1997), and Kohl *et al.* (1991) cannot be held to make the present invention obvious. Reversal of the Examiner's rejection of claim 1 is therefore respectfully requested.

Respectfully submitted,



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VIII. Claims Appendix

Claim 1: A method for decreasing the signs and symptoms of motion sickness comprising administering to a subject an effective amount of a halogenated pheniramine to decrease the signs or symptoms of motion sickness, wherein said halogenated pheniramine consists of chlorpheniramine or enantiomers thereof, wherein said halogenated pheniramine is administered orally or topically, and wherein said halogenated pheniramine is administered at a dose of 12 mg.

IX. Evidence Appendix

Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 10th edition. 2001. J.G. Hardman and L.E. Limbird (eds.), McGraw Hill: New York, chapter 25.

Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 10th edition. 2001. J.G. Hardman and L.E. Limbird (eds.), McGraw Hill: New York, see page 39 of chapter 2.

Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 10th edition. 2001. J.G. Hardman and L.E. Limbird (eds.), McGraw Hill: New York, chapter 1.

CHAPTER 25

HISTAMINE, BRADYKININ, AND THEIR ANTAGONISTS

Nancy J. Brown and L. Jackson Roberts II

This chapter describes the physiological role and pathophysical consequences of histamine release and provides a summary of the therapeutic use of histamine H_1-receptor antagonists. H_2-receptor antagonists are discussed in detail in Chapter 37 in the context of prevention and treatment of peptic ulcers, their principal therapeutic application. The identity and role of H_2-receptor subtypes are described briefly, as are the newly developed H_3 agonists and antagonists, although none has been approved by the U.S. Food and Drug Administration (FDA) for clinical use to date.

The second part of the chapter describes the physiology and pathophysiology of the kinins and kallidins, a subset of autacoids that contribute to the inflammatory response. The identification of at least two distinct receptors for kinins, designated B_1 and B_2, allows for the development of selective receptor antagonists, which also are discussed. Serotonin (5-hydroxytryptamine; 5-HT), another autacoid often considered in the same context as histamine and the kinin and kallidin agents, is discussed in detail in Chapter 11.

HISTAMINE

History. The history of β-aminoethylimidazole, or histamine, parallels that of acetylcholine (ACh). Both compounds were synthesized as chemical curiosities before their biological significance was recognized; both were first detected as uterine stimulants in extracts of ergot, from which they were subsequently isolated; and both proved to be contaminants of ergot that resulted from bacterial action.

When Dale and Laidlaw (1910, 1911) subjected histamine to intensive pharmacological study, they discovered that it stimulated a host of smooth muscles and had an intense vasodepressor action. Remarkably, they pointed out that the immediate signs displayed by a sensitized animal when injected with a normally inert protein closely resemble those of poisoning by histamine. These comments anticipated by many years the discovery of the presence of histamine in the body and its release during immediate hypersensitivity reactions and upon cellular injury. It was not until 1927 that Best *et al.* isolated histamine from very fresh samples of liver and lung, thereby establishing that this amine is a natural constituent of the body. Demonstrations of its presence in a variety of other tissues soon followed—hence the name *histamine* after the Greek word for tissue, *histos*.

Meanwhile, Lewis and his colleagues had amassed evidence that a substance with the properties of histamine ("H-substance") was liberated from the cells of the skin by injurious stimuli, including the reaction of antigen with antibody (Lewis, 1927). Given the chemical evidence of histamine's presence in the body, there remained little impediment to supposing that Lewis' "H-substance" was histamine itself. It is now evident that endogenous histamine plays a role in the immediate allergic response and is an important regulator of gastric acid secretion. More recently, a role for histamine as a modulator of neurotransmitter release in the central and peripheral nervous systems also has emerged.

Early suspicions that histamine acts through more than one receptor have been borne out, and it is clear that there are at least three distinct classes of receptors for histamine, designated H_1 (Ash and Schild, 1966), H_2 (Black *et al.*, 1972), and H_3 (Arrang *et al.*, 1983). H_1 receptors are blocked selectively by the classical "antihistamines" (such as pyrilamine) developed around 1940. H_2-receptor antagonists were introduced in the early 1970s. The discovery of H_2 antagonists has contributed greatly to the resurgence of interest in histamine in biology and clinical medicine (*see* Chapter 37). H_3 receptors were originally discovered as a presynaptic autoreceptor on histamine-containing neurons that mediate feedback inhibition of the release and synthesis of histamine. The recent development of selective H_3-receptor agonists and antagonists has led to an increased understanding of the importance of H_3 receptors in histaminergic neurons *in vivo*. None of these H_3-receptor agonists or antagonists, however, has yet emerged as a therapeutic agent. Renewed interest in clinical use of H_1-receptor antagonists has occurred over the past 15 years due to the development of second-generation antagonists, collectively referred to as *nonsedating antihistamines*.

Chemistry. Histamine is a hydrophilic molecule comprising an imidazole ring and an amino group connected by two methylene groups. The pharmacologically active form at all histamine receptors is the monocationic Nγ—H tautomer—that is, the charged form of the species depicted in Figure 25–1, although

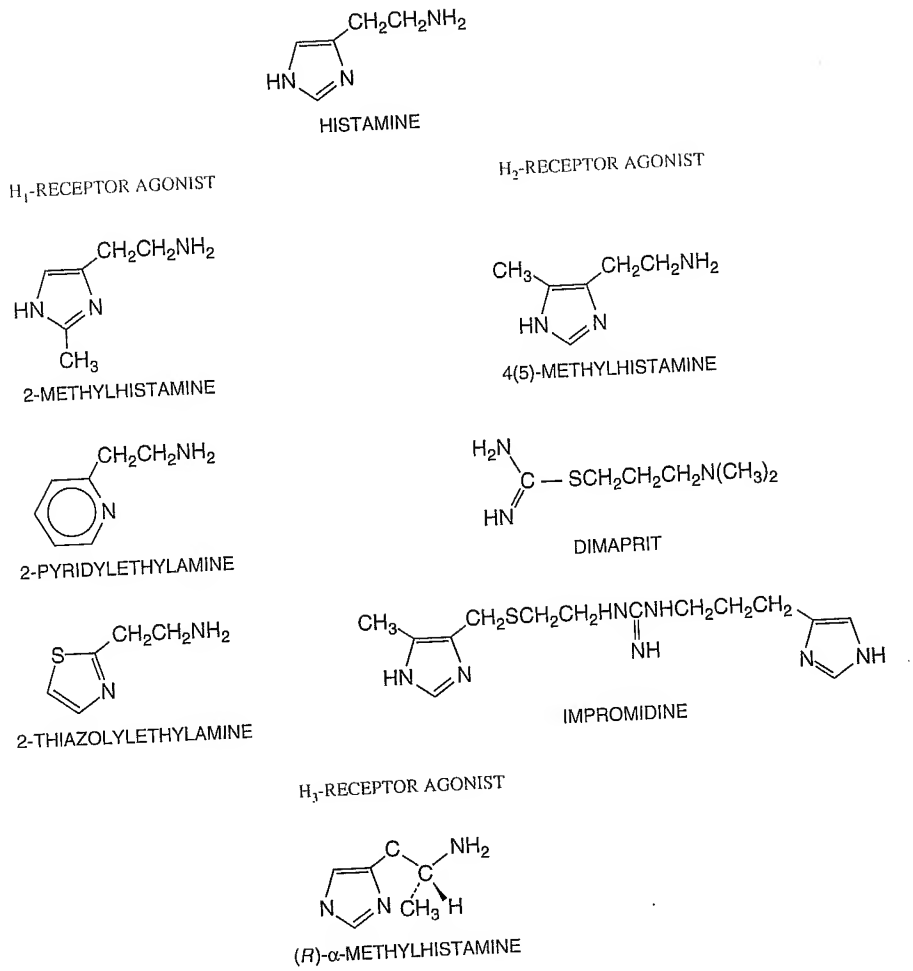


Figure 25–1. Structure of histamine and some H_1, H_2, and H_3 agonists.

different chemical properties of this monocation may be involved in interactions with the H_1 and H_2 receptors (Ganellin, in Ganellin and Parsons, 1982). The three classes of histamine receptors can be activated differently by analogs of histamine (*see* Figure 25–1). Thus, 2-methylhistamine preferentially elicits responses mediated by H_1 receptors, whereas 4(5)-methylhistamine has a preferential effect on H_2 receptors (Black *et al.*, 1972). A chiral analog of histamine with restricted conformational freedom, (*R*)-α-methylhistamine, is the preferred agonist at H_3-receptor sites (Arrang *et al.*, 1987).

Distribution and Biosynthesis of Histamine

Distribution. Histamine is widely, if unevenly, distributed throughout the animal kingdom and is present in many venoms, bacteria, and plants. Almost all mammalian tissues contain histamine in amounts ranging from less than 1 μg/g to more than 100 μg/g. Concentrations in plasma and other body fluids generally are very low, but human cerebrospinal fluid contains significant amounts. The mast cell is the predominant storage site for histamine in most tissues (*see* below); the concentration of histamine is particularly high in tissues that contain large numbers of mast cells, such as skin, the mucosa of the bronchial tree, and the intestinal mucosa. However, some tissues synthesize and turn over histamine at a remarkably fast rate, even though their steady-state content of the amine may be modest.

Synthesis, Storage, and Metabolism. Histamine, in the amounts normally ingested or formed by bacteria in the gastrointestinal tract, is rapidly metabolized and eliminated in the urine. Every mammalian tissue that contains histamine is capable of synthesizing it from histidine by virtue of its content of L-histidine decarboxylase. The chief site of histamine storage in most tissues is the mast cell; in the blood, it is the basophil. These cells synthesize histamine and store it in secretory granules. At the secretory granule pH of ~5.5, histamine is positively charged and ionically complexed with negatively charged acidic groups on other secretory granule constituents, primarily proteases and heparin or chondroitin sulfate proteoglycans (Serafin and Austen, 1987). The turnover rate of histamine in secretory granules is slow, and when tissues rich in mast cells are depleted of their stores of histamine, it may take weeks before concentrations of the autacoid return to normal levels. Non-mast-cell sites of histamine formation or storage include cells

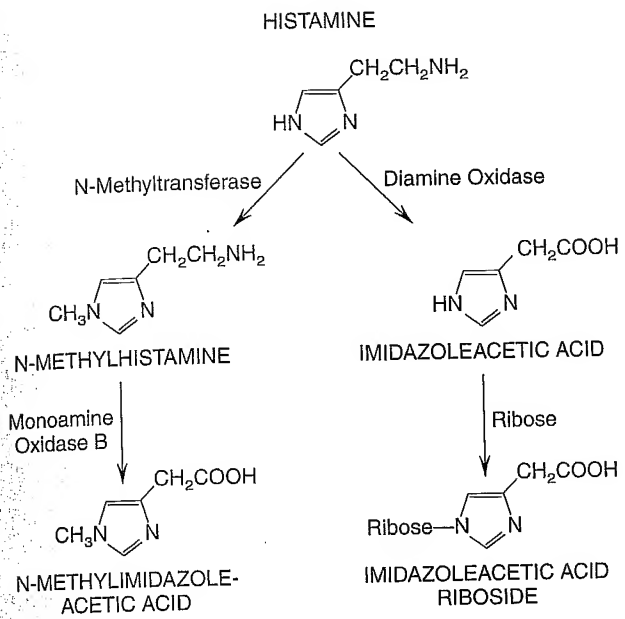


Figure 25–2. Pathways of histamine metabolism in human beings.

See text for further explanation.

of the epidermis, cells in the gastric mucosa, neurons within the central nervous system (CNS), and cells in regenerating or rapidly growing tissues. Turnover is rapid at these sites, since the histamine is continuously released rather than stored. Non-mast-cell sites of histamine production contribute significantly to the daily excretion of histamine and its metabolites in the urine. Since L-histidine decarboxylase is an inducible enzyme, the histamine-forming capacity at such non-mast-cell sites is subject to regulation by various physiological and pathophysiological factors.

There are two major paths of histamine metabolism in human beings (Figure 25–2). The more important of these involves ring methylation to form *N*-methylhistamine. This is catalyzed by histamine-*N*-methyltransferase, which is widely distributed. Most of the *N*-methylhistamine formed is then converted by monoamine oxidase (MAO) to *N*-methylimidazoleacetic acid. This reaction can be blocked by MAO inhibitors (*see* Chapter 19). Alternatively, histamine undergoes oxidative deamination catalyzed mainly by the nonspecific enzyme diamine oxidase (DAO), yielding imidazoleacetic acid, which is then converted to imidazoleacetic acid riboside. These metabolites have little or no activity and are excreted in the urine. One important aspect regarding these metabolites, however, is that it has been shown that measurement of *N*-methylhistamine in urine affords a more reliable index of endogenous histamine production than does measurement of histamine, because it circumvents the problem of artifactually elevated levels of histamine in urine that can arise from the ability of some genitourinary tract bacteria to decarboxylate histidine (Roberts and Oates, 1991). In addition, the metabolism of histamine appears to be altered in patients with mastocytosis such that measurement of histamine metabolites has been shown to be a more sensitive diagnostic indicator of the disease than is measurement of histamine (Keyzer *et al.*, 1983).

Functions of Endogenous Histamine

Histamine has important physiological roles. Because histamine is one of the preformed mediators stored in the mast cell, its release as a result of the interaction of antigen with IgE antibodies on the mast cell surface plays a central role in immediate hypersensitivity and allergic responses. The actions of histamine on bronchial smooth muscle and blood vessels account in part for the symptoms of the allergic response. In addition, certain clinically useful drugs can act directly on mast cells to release histamine, thereby explaining some of their untoward effects. Histamine has a major role in the regulation of gastric acid secretion, and its function as a modulator of neurotransmitter release has recently become appreciated.

Role in Allergic Responses. The principal target cells of immediate hypersensitivity reactions are mast cells and basophils (Galli, 1993; Schwartz, 1994). As part of the allergic response to an antigen, reaginic antibodies (IgE) are generated and bind to the surface of mast cells and basophils *via* high-affinity F_c receptors that are specific for IgE. This receptor, FcεRI, consists of α, β, and two γ chains, all of which have been molecularly characterized (Ravetch and Kinet, 1991). The IgE molecules function as receptors for antigens, and *via* FcεRI, interact with signal transduction systems in the membranes of sensitized cells. Atopic individuals, as opposed to those who are not, develop IgE antibodies to commonly inhaled antigens. This is a heritable trait, and a candidate gene has been identified (Cookson *et al.*, 1992; Shirakawa *et al.*, 1994). Since the candidate gene encodes the β-chain of FcεRI, an even greater interest has been generated for understanding the transmembrane signaling mechanisms of mast cells and basophils. Upon exposure, antigen bridges the IgE molecules and causes activation of tyrosine kinases and subsequent phosphorylation of multiple protein substrates within 5 to 15 seconds after contact with antigen (Scharenberg and Kinet in Symposium, 1994). Kinases implicated in this event include the *src*-related kinases lyn and syk. Prominent among the newly phosphorylated proteins are the β and γ subunits of the FcεRI itself and phospholipase Cγ1 and Cγ2. Subsequently, inositol phospholipids are metabolized, with a result being the release of Ca^{2+} from intracellular stores, thereby raising free cytosolic Ca^{2+} levels (*see* Chapter 2). These events trigger the extrusion of the contents of secretory granules by exocytosis. The secretory behavior of mast cells and basophils is similar to that of various endocrine and exocrine glands and conforms to a general pattern of stimulus-secretion coupling in which a secretagogue-induced rise in the intracellular concentration of Ca^{2+} serves to initiate exocytosis. The mechanism by which the rise in Ca^{2+} leads to fusion of the secretory granule with the plasma membrane is not fully elucidated, but is likely to involve activation of Ca^{2+}/calmodulin-dependent protein kinases and protein kinase C.

Release of Other Autacoids. The release of histamine provides only a partial explanation for all of the biological effects that ensue from immediate hypersensitivity reactions. This is because a broad spectrum of other inflammatory mediators is released upon mast cell activation.

In addition to activation of phospholipase C and the hydrolysis of inositol phospholipids, stimulation of IgE receptors also activates phospholipase A_2, leading to the production of a host of mediators, including platelet-activating factor (PAF) and metabolites of arachidonic acid. Leukotriene D_4, which is generated in this way, is a potent contractor of the smooth muscle of the bronchial tree (*see* Chapters 26 and 28). Kinins also are generated during some allergic responses (*see* below). Thus, the mast cell secretes a variety of inflammatory compounds in addition to histamine, and each contributes to varying extents to the major symptoms of the allergic response: constriction of the bronchi, decrease in blood pressure, increased capillary permeability, and edema formation (*see* below).

Regulation of Mediator Release. The wide variety of mediators released during the allergic response explains the ineffectiveness of drug therapy focused on a single mediator. Considerable emphasis has been placed on the regulation of mediator release from mast cells and basophils, and these cells do contain receptors linked to signaling systems that can enhance or block the IgE-induced release of mediators.

Agents that act at muscarinic or α-adrenergic receptors enhance the release of mediators, although this effect is of little clinical significance. Effective inhibition of the secretory response can be achieved with epinephrine and related drugs that act through β_2-adrenergic receptors. The effect is the result of accumulation of cyclic AMP. However, the beneficial effects of β-adrenergic agonists in allergic states such as asthma are due mainly to their relaxant effect on bronchial smooth muscle (*see* Chapters 10 and 28). Cromolyn sodium owes its clinical utility to its capacity to inhibit the release of mediators from mast and other cells in the lung (*see* Chapter 28).

Histamine Release by Drugs, Peptides, Venoms, and Other Agents. Many compounds, including a large number of therapeutic agents, stimulate the release of histamine from mast cells directly and without prior sensitization. Responses of this sort are most likely to occur following intravenous injections of certain categories of substances, particularly those that are organic bases. Among these bases are amides, amidines, quaternary ammonium compounds, pyridinium compounds, piperidines, alkaloids, and antibiotic bases. Tubocurarine, succinylcholine, morphine, radiocontrast media, and certain carbohydrate plasma expanders also may elicit the response. The phenomenon is one of clinical concern, for it may account for unexpected anaphylactoid reactions. Vancomycin-induced "red-man syndrome" involving upper body and facial flushing and hypotension may be mediated, at least in part if not entirely, through histamine release (Levy *et al.,* 1987).

In addition to therapeutic agents, certain experimental compounds stimulate the release of histamine as their dominant pharmacological characteristic. The archetype is the polybasic substance known as compound 48/80. This is a mixture of low-molecular-weight polymers of *p*-methoxy-*N*-methylphenethylamine, of which the hexamer is most active (*see* Lagunoff *et al.,* 1983).

Basic polypeptides often are effective histamine releasers, and their potency generally increases with the number of basic groups over a limited range. Polymyxin B is very active; others include bradykinin and substance P. Since basic polypeptides are released upon tissue injury or are present in venoms, they constitute pathophysiological stimuli to secretion for mast cells and basophils. Anaphylotoxins (C3a and C5a), which are low-molecular-weight peptides that are cleaved from the complement system, may act similarly.

Within seconds of the intravenous injection of a histamine liberator, human subjects experience a burning, itching sensation. This effect, most marked in the palms of the hand and in the face, scalp, and ears, is soon followed by a feeling of intense warmth. The skin reddens, and the color rapidly spreads over the trunk. Blood pressure falls, the heart rate accelerates, and the subject usually complains of headache. After a few minutes, blood pressure recovers, and crops of hives usually appear on the skin. Colic, nausea, hypersecretion of acid, and moderate bronchospasm also occur frequently. The effect becomes less intense with successive injections as the mast-cell stores of histamine are depleted. Histamine liberators do not deplete tissues of non-mast-cell histamine.

Mechanism. All of the above-mentioned histamine-releasing substances can activate the secretory response of mast cells or basophils by causing a rise in intracellular Ca^{2+}. Some are ionophores and transport Ca^{2+} into the cell; others, such as the anaphylotoxins, appear to act like specific antigens to increase membrane permeability to Ca^{2+}. Still others, such as mastoparan (a peptide from wasp venom), may bypass cell-surface receptors and directly stimulate guanine nucleotide–binding regulatory proteins (G proteins), which then activate phospholipase C (Higashijima *et al.,* 1988). Basic histamine releasers, such as compound 48/80 and polymyxin B, act principally by mobilizing Ca^{2+} from cellular stores (*see* Lagunoff *et al.,* 1983).

Histamine Release by Other Means. Clinical conditions in which release of histamine occurs in response to other stimuli include cold urticaria, cholinergic urticaria, and solar urticaria. Some of these involve specific secretory responses of the mast cells and, indeed, cell-fixed IgE. However, histamine release also occurs whenever there is nonspecific cell damage from any cause. The redness and urticaria that follow scratching of the skin is a familiar example.

Gastric Carcinoid Tumors and Increased Proliferation of Mast Cells and Basophils. In urticaria pigmentosa (cutaneous mastocytosis), mast cells aggregate in the upper corium and give rise to pigmented cutaneous lesions that urticate when stroked. In systemic mastocytosis, overproliferation of mast cells also is found in other organs. Patients with these syndromes suffer a constellation of signs and symptoms attributable to excessive histamine release, including urticaria, dermographism, pruritus, headache, weakness, hypotension, flushing of the face, and a variety of gastrointestinal effects such as peptic ulceration. Episodes of mast cell activation with attendant systemic histamine release are precipitated by a variety of stimuli, including exertion, emotional upset, and exposure to heat, and from exposure to drugs that release histamine directly or to which patients are allergic. In myelogenous leukemia, excessive numbers of basophils are present in the blood raising its histamine content to high levels, which may contribute to chronic pruritus.

Gastric carcinoid tumors secrete histamine, which is responsible for episodes of vasodilation and contributes to the patchy "geographical" flush (Roberts *et al.,* 1979).

Gastric Acid Secretion. Histamine is a powerful gastric secretagogue and evokes a copious secretion of acid from parietal cells by acting on H_2 receptors. The output of pepsin and intrinsic factor also is increased. However, the secretion of acid also is evoked by stimulation of the vagus nerve and by the enteric hormone gastrin. In addition, there appear to be cells in the gastric mucosa that contain somatostatin, which can inhibit secretion of acid by parietal cells; the release of somatostatin is inhibited by acetylcholine. The interplay among these endogenous regulators has not been precisely defined. However, it is clear that histamine is the dominant physiological mediator of acid secretion because blockade of H_2 receptors can not only eradicate acid secretion in response to histamine, but also cause nearly complete inhibition of responses to gastrin or vagal stimulation. This is discussed in more detail in Chapter 37.

Central Nervous System. There is substantial evidence that histamine functions as a neurotransmitter in the CNS. Histamine, histidine decarboxylase, and enzymes that catalyze the degradation of histamine are distributed nonuniformly in the CNS and are concentrated in synaptosomal fractions of brain homogenates. H_1 receptors are found throughout the CNS and are densely concentrated in the hypothalamus. Histamine increases wakefulness *via* H_1 receptors (Monti, 1993), explaining the potential for sedation by classical antihistamines. Histamine acting through H_1 receptors inhibits appetite (Ookuma *et al.,* 1993). Histamine-containing neurons may participate in the regulation of drinking, body temperature, and the secretion of antidiuretic hormone, as well as in the control of blood pressure and the perception of pain. Both H_1 and H_2 receptors seem to be involved in these responses (*see* Hough, 1988).

Pharmacological Effects: H_1 and H_2 Receptors

Once released, histamine can exert local or widespread effects on smooth muscles and glands. The autacoid contracts many smooth muscles, such as those of the bronchi and gut, but powerfully relaxes others, including those of small blood vessels. It also is a potent stimulus to gastric acid secretion. Effects attributable to these actions dominate the overall response to histamine; however, there are other effects, such as formation of edema and stimulation of sensory nerve endings. Many of these effects, such as bronchoconstriction and contraction of the gut, are mediated by H_1 receptors (Ash and Schild, 1966). Other effects, most notably gastric secretion, are the results of activation of H_2 receptors and, accordingly, can be inhibited by H_2-receptor antagonists (Black *et al.,* 1972). Some responses, such as the hypotension that results from vascular dilation, are mediated by both H_1 and H_2 receptors.

Histamine Toxicity from Ingestion. Histamine has been identified as the toxin in food poisoning from spoiled scombroid fish, such as tuna (Morrow *et al.,* 1991). Bacteria in spoiled scombroid fish, which have a high histidine content, decarboxylate histidine to form large quantities of histamine. Ingestion of the fish causes severe nausea, vomiting, headache, flushing, and sweating. Histamine toxicity, manifested by headache and other symptoms, also can be seen following red wine consumption in persons who possibly have a diminished ability to degrade histamine (Wantke *et al.,* 1994). The symptoms of histamine poisoning can be suppressed by H_1 receptor antagonists.

Cardiovascular System. Histamine characteristically causes dilation of small blood vessels, resulting in flushing, lowered total peripheral resistance, and a fall in systemic blood pressure. In addition, histamine tends to increase capillary permeability.

Vasodilation. This is the characteristic action of histamine on the vasculature, and it is by far the most important vascular effect of histamine in human beings. Vasodilation involves both H_1 and H_2 receptors distributed throughout the resistance vessels in most vascular beds; however, quantitative differences are apparent in the degree of dilation that occurs in various beds. Activation of either the H_1 or H_2 type of histamine receptor can elicit maximal vasodilation, but the responses differ in their sensitivity to histamine, in the duration of the effect, and in the mechanism of their production. H_1 receptors have the higher affinity for histamine and mediate a dilator response that is relatively rapid in onset and short lived. By contrast, activation of H_2 receptors causes dilation that develops more slowly and is more sustained. As a result, H_1 antagonists effectively counter small dilator responses to low concentrations of histamine but only blunt the initial phase of larger responses to higher concentrations of the amine. H_2 receptors are located on vascular smooth muscle cells, and the vasodilator effects produced by their stimulation are mediated by cyclic AMP; H_1 receptors reside on endothelial cells, and their stimulation leads to the formation of local vasodilator substances (*see* below).

Increased "Capillary" Permeability. This classical effect of histamine on small vessels results in outward passage of plasma protein and fluid into the extracellular spaces, an increase in the flow of lymph and its protein content, and formation of edema. H_1 receptors clearly are important for this response; whether or not H_2 receptors also participate is uncertain.

Increased permeability results mainly from actions of histamine on postcapillary venules, where histamine causes the endothelial cells to contract and separate at their boundaries and thus to expose the basement membrane, which is freely permeable to plasma protein and

fluid. The gaps between endothelial cells also may permit passage of circulating cells that are recruited to the tissues during the mast-cell response. Recruitment of circulating leukocytes is promoted by H_1-receptor–mediated upregulation of leukocyte adhesion. This process involves histamine-induced expression of the adhesion molecule P-selectin on the endothelial cells (Gaboury *et al.*, 1995).

Triple Response. If histamine is injected intradermally, it elicits a characteristic phenomenon known as the "triple response" (Lewis, 1927). This consists of (1) a localized red spot, extending for a few millimeters around the site of injection, that appears within a few seconds and reaches a maximum in about a minute; (2) a brighter red flush, or "flare," extending about 1 cm or so beyond the original red spot and developing more slowly; and (3) a wheal that is discernible in 1 to 2 minutes and occupies the same area as the original small red spot at the injection site. The red spot results from the direct vasodilatory effect of histamine, the flare is due to histamine-induced stimulation of axon reflexes that cause vasodilation indirectly, and the wheal reflects histamine's capacity to increase capillary permeability.

Constriction of Larger Vessels. Histamine tends to constrict larger blood vessels, in some species more than in others. In rodents, the effect extends to the level of the arterioles and may overshadow dilation of the finer blood vessels. A net increase in total peripheral resistance and an elevation of blood pressure can be observed.

Heart. Histamine has direct actions on the heart that affect both contractility and electrical events. It increases the force of contraction of both atrial and ventricular muscle by promoting the influx of Ca^{2+}, and it speeds heart rate by hastening diastolic depolarization in the SA node. It also acts directly to slow AV conduction, to increase automaticity, and, in high doses especially, to elicit arrhythmias. With the exception of slowed AV conduction, which involves mainly H_1 receptors, all these effects are largely attributable to H_2 receptors. If histamine is given intravenously, direct cardiac effects of histamine are not prominent and are overshadowed by baroreceptor reflexes elicited by the reduced blood pressure.

Histamine Shock. Histamine given in large doses or released during systemic anaphylaxis causes a profound and progressive fall in blood pressure. As the small blood vessels dilate, they trap large amounts of blood, and as their permeability increases, plasma escapes from the circulation. Resembling surgical or traumatic shock, these effects diminish effective blood volume, reduce venous return, and greatly lower cardiac output.

Extravascular Smooth Muscle. Histamine stimulates, or more rarely relaxes, various smooth muscles. Contraction is due to activation of H_1 receptors and relaxation (for the most part) to activation of H_2 receptors. Responses vary widely, even in individuals (*see* Parsons, in Ganellin and Parsons, 1982). Bronchial muscle of guinea pigs is exquisitely sensitive. Minute doses of histamine also will evoke intense bronchoconstriction in patients with bronchial asthma and certain other pulmonary diseases; in normal human beings the effect is much less pronounced. Although the spasmogenic influence of H_1 receptors is dominant in human bronchial muscle, H_2 receptors with dilator function also are present. Thus, histamine-induced bronchospasm *in vitro* is potentiated slightly by H_2 blockade. In asthmatic subjects in particular, histamine-induced bronchospasm may involve an additional, reflex component that arises from irritation of afferent vagal nerve endings (*see* Eyre and Chand, in Ganellin and Parsons, 1982; Nadel and Barnes, 1984).

The uterus of some species contracts to histamine; in the human uterus, gravid or not, the response is negligible. Responses of intestinal muscle also vary with species and region, but the classical effect is contraction. Bladder, ureter, gallbladder, iris, and many other smooth muscle preparations are affected little or inconsistently by histamine.

Exocrine Glands. As mentioned above, histamine is an important physiological regulator of gastric acid secretion. This effect is mediated by H_2 receptors (*see* Chapter 37).

Nerve Endings: Pain, Itch, and Indirect Effects. Histamine stimulates various nerve endings. Thus, when released in the epidermis, it causes itch; in the dermis, it evokes pain, sometimes accompanied by itching. Stimulant actions on one or another type of nerve ending, including autonomic afferents and efferents, have been mentioned above as factors that contribute to the "flare" component of the triple response and to indirect effects of histamine on the bronchi and other organs. In the periphery, neuronal receptors for histamine are generally of the H_1 type (*see* Rocha e Silva, 1978; Ganellin and Parsons, 1982).

Mechanism of Action. The H_1 and H_2 receptors have been cloned and shown to belong to the superfamily of G protein–coupled receptors. H_1 receptors are coupled to phospholipase C, and their activation leads to formation of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerols from phospholipids in the cell membrane; IP_3 causes a rapid release of Ca^{2+} from the endoplasmic reticulum. Diacylglycerols (and Ca^{2+}) activate protein kinase C, while Ca^{2+} activates Ca^{2+}/calmodulin-dependent protein kinases and phospholipase A_2 in the target cell to generate the characteristic response. H_2 receptors are linked to the stimulation of adenylyl cyclase and thus to the activation of cyclic AMP–dependent protein kinase in the target cell. In a species-dependent manner, adenosine receptors may interact with H_1 receptors. In the CNS of human beings, activation of adenosine A_1 receptors inhibits second messenger generation *via* H_1 receptors. A possible mechanism for this is interaction (termed *cross-talk*) between the G proteins to which the A_1 and H_1 receptors are coupled functionally (Dickenson and Hill, 1993).

In the smooth muscle of large blood vessels, bronchi, and intestine, the stimulation of H_1 receptors and the resultant IP_3-mediated release of intracellular Ca^{2+} leads to activation of the Ca^{2+}/calmodulin-dependent myosin light chain kinase.

This enzyme phosphorylates the 20,000 dalton myosin light chain, with resultant enhancement of cross-bridge cycling and contraction. The effects of histamine on sensory nerves also are mediated by H_1 receptors.

As mentioned above, the vasodilator effects of histamine are mediated by both H_1 and H_2 receptors that are located on different cell types in the vascular bed: H_1 receptors on the vascular endothelial cells and H_2 receptors on smooth muscle cells. Activation of H_1 receptors leads to increased intracellular Ca^{2+}, activation of phospholipase A_2, and the local production of endothelium-derived relaxing factor, which is nitric oxide (Palmer *et al.*, 1987). Nitric oxide diffuses to the smooth muscle cell, where it activates a soluble guanylyl cyclase and causes the accumulation of cyclic GMP. Stimulation of a cyclic GMP–dependent protein kinase and a decrease in intracellular Ca^{2+} are thought to be involved in the relaxation caused by this cyclic nucleotide. The activation of phospholipase A_2 in endothelial cells also leads to the formation of prostaglandins, predominantly prostacyclin (PGI_2); this vasodilator makes an important contribution to endothelium-mediated vasodilation in some vascular beds.

The mechanism of cyclic AMP–mediated relaxation of smooth muscle is not entirely clear, but it is presumed to involve a decrease in intracellular Ca^{2+} (*see* Taylor *et al.*, 1989). Cyclic AMP–mediated actions in the heart, mast cells, basophils, and other tissues also are understood incompletely, but the effects of histamine that are mediated by H_2 receptors obviously would be produced in the same fashion as those resulting from stimulation of β-adrenergic receptors or other receptors that are linked to the activation of adenylyl cyclase.

Clinical Uses

The practical applications of histamine are limited to uses as a diagnostic agent. Histamine (*histamine phosphate*) is used to assess nonspecific bronchial hyperreactivity in asthmatics and as a positive control injection during allergy skin testing.

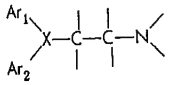
H_1-RECEPTOR ANTAGONISTS

Although antagonists that act selectively at the three types of histamine receptors have been developed, this discussion is confined to the properties and clinical uses of H_1 antagonists. Specific H_2 antagonists (*e.g.*, cimetidine, ranitidine) are used extensively in the treatment of peptic ulcers; these are discussed in Chapter 37. The properties of agonists and antagonists at H_3 receptors are discussed later in this chapter. Such agents are not yet available for clinical use.

History. Histamine-blocking activity was first detected in 1937 by Bovet and Staub in one of a series of amines with a phenolic ether function. The substance, 2-isopropyl-5-methylphenoxyethyldiethyl-amine, protected guinea pigs against several lethal doses of histamine, antagonized histamine-induced spasm of various smooth muscles, and lessened the symptoms of anaphylactic shock. This drug was too toxic for clinical use, but by 1944, Bovet and his colleagues had described pyrilamine maleate, which is still one of the most specific and effective histamine antagonists of this category. The discovery of the highly effective histamine antagonists *diphenhydramine* and *tripelennamine* soon followed (*see* Bovet, 1950; Ganellin, in Ganellin and Parsons, 1982). In the 1980s, nonsedating H_1-histamine–receptor antagonists were developed for treatment of allergic diseases.

By the early 1950s, many compounds with histamine-blocking activity were available to physicians, but they uniformly failed to inhibit certain responses to histamine, most conspicuously gastric acid secretion. The discovery by Black and colleagues of a new class of drugs that blocked histamine-induced gastric acid secretion provided new pharmacological tools with which to explore the functions of endogenous histamine. This discovery ushered in a major new class of therapeutic agents, the H_2 receptor antagonists, including *cimetidine* (TAGAMET), *famotidine* (PEPCID), *nizatidine* (AXID), and *ranitidine* (ZANTAC) (*see* Chapter 37).

Structure–Activity Relationship. All of the available H_1 receptor antagonists are reversible, competitive inhibitors of the interaction of histamine with H_1 receptors. Like histamine, many H_1 antagonists contain a substituted ethylamine moiety,  . Unlike histamine, which has a primary amino group and a single aromatic ring, most H_1 antagonists have a tertiary amino group linked by a two- or three-atom chain to two aromatic substituents and conform to the general formula:



where Ar is aryl and X is a nitrogen or carbon atom or a —C—O— ether linkage to the beta-aminoethyl side chain. Sometimes the two aromatic rings are bridged, as in the tricyclic derivatives, or the ethylamine may be part of a ring structure (Figure 25–3). (*see* Ganellin, in Ganellin and Parsons, 1982.)

Pharmacological Properties

Most H_1 antagonists have similar pharmacological actions and therapeutic applications and can be discussed together conveniently. Their effects are largely predictable from knowledge of the responses to histamine that involve interaction with H_1 receptors.

Smooth Muscle. H_1 antagonists inhibit most responses of smooth muscle to histamine. Antagonism of the constrictor action of histamine on respiratory smooth muscle is easily shown *in vivo* or *in vitro*. In guinea pigs, for example, death by asphyxia follows quite small doses of histamine, yet the animal may survive a hundred lethal

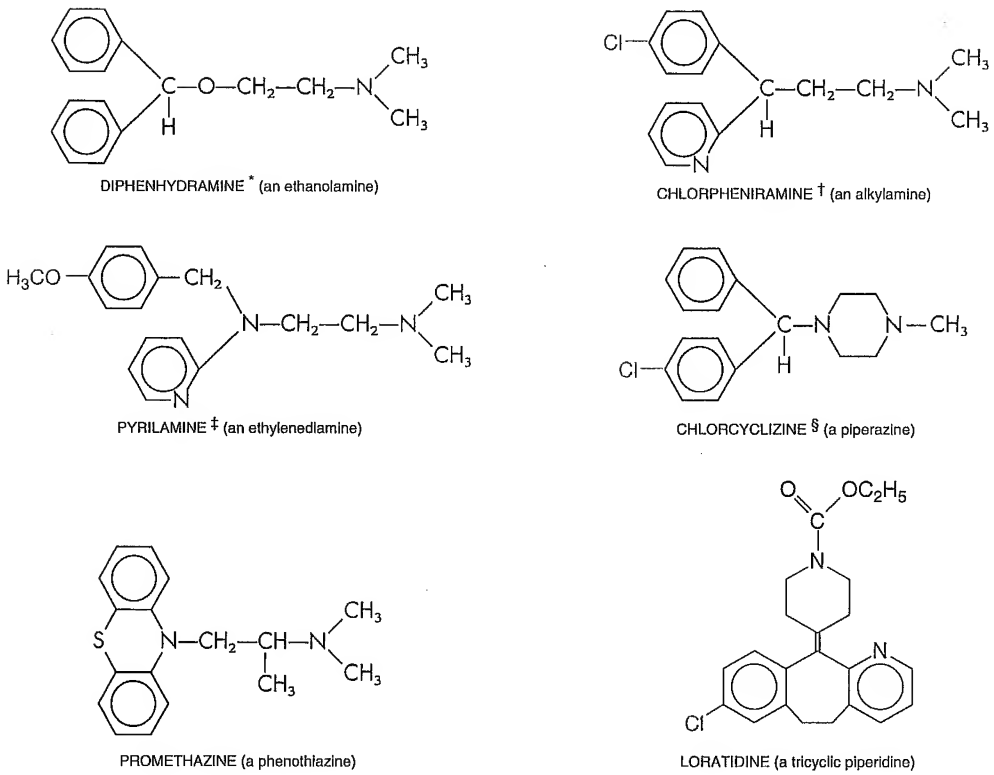


Figure 25–3. Representative H_1 antagonists.

*Dimenhydrinate is a combination of diphenhydramine and 8-chlorotheophylline in equal molecular proportions.
†Pheniramine is the same less Cl.
‡Tripelennamine is the same less H_3CO.
§Cyclizine is the same less Cl.

doses of histamine if given an H_1 antagonist. In the same species, striking protection also is afforded against anaphylactic bronchospasm. This is not so in human beings, where allergic bronchoconstriction appears to be caused by a variety of mediators such as leukotrienes and platelet activating factor (*see* Chapter 26).

Within the vascular tree, the H_1 antagonists inhibit both the vasoconstrictor effects of histamine and, to a degree, the more rapid vasodilator effects that are mediated by H_1 receptors on endothelial cells. Residual vasodilation reflects the involvement of H_2 receptors on smooth muscle and can be suppressed only by the concurrent administration of an H_2 antagonist. Effects of the histamine antagonists on histamine-induced changes in systemic blood pressure parallel these vascular effects.

Capillary Permeability. H_1 antagonists strongly block the action of histamine that results in increased capillary permeability and formation of edema and wheal.

Flare and Itch. The flare component of the triple response and the itching caused by intradermal injection of histamine are two different manifestations of the action of histamine on nerve endings. H_1 antagonists suppress both.

Exocrine Glands. Gastric secretion is not inhibited at all by H_1 antagonists, and they suppress histamine-evoked salivary, lacrimal, and other exocrine secretions with variable responses. The atropine-like properties of many of these agents, however, may contribute to lessened secretion in cholinergically innervated glands and reduce ongoing secretion in, for example, the respiratory tree.

Immediate Hypersensitivity Reactions: Anaphylaxis and Allergy. During hypersensitivity reactions, histamine is one of many potent autacoids released (*see* above), and its relative contribution to the ensuing symptoms varies widely with species and tissue. The protection afforded by histamine antagonists thus also varies accordingly. In human beings, some phenomena, such as edema formation and itch, are effectively suppressed. Others, such as

hypotension, are less so. This may be explained by the existence of other mast-cell mediators, specifically prostaglandin D_2, also contributing to the vasodilation (Roberts *et al.*, 1980). Bronchoconstriction is reduced little, if at all (*see* Dahlén *et al.*, 1983).

Central Nervous System. The first-generation H_1 antagonists can both stimulate and depress the CNS. Stimulation occasionally is encountered in patients given conventional doses, who become restless, nervous, and unable to sleep. Central excitation also is a striking feature of poisoning, which commonly results in convulsions, particularly in infants. Central depression, on the other hand, is the usual accompaniment of therapeutic doses of the older H_1 antagonists. Diminished alertness, slowed reaction times, and somnolence are common manifestations. Some of the H_1 antagonists are more likely to depress the CNS than others, and patients vary in their susceptibility and responses to individual drugs. The ethanolamines (*e.g.*, diphenhydramine; *see* Figure 25–3) are particularly prone to cause sedation.

The second-generation ("nonsedating") H_1 antagonists (*e.g., loratadine, cetirizine, fexofenadine*) are largely excluded from the brain when given in therapeutic doses, because they do not cross the blood–brain barrier appreciably. Their effects on objective measures of sedation such as sleep latency, EEG, and standardized performance tests are similar to those of placebo (Simons and Simons, 1994). Because of the sedation that occurs with first-generation antihistamines, these drugs cannot be tolerated or used safely by many patients. Thus, the availability of nonsedating antihistamines has been an important advance that allows the general use of these agents.

An interesting and useful property of certain H_1 antagonists is the capacity to counter motion sickness. This effect was first observed with *dimenhydrinate* and subsequently with *diphenhydramine* (the active moiety of dimenhydrinate), various piperazine derivatives, and *promethazine*. The latter drug has perhaps the strongest muscarinic blocking activity among these agents and is among the most effective of the H_1 antagonists in combating motion sickness (*see* below). Since scopolamine is the most potent drug for the prevention of motion sickness (*see* Chapter 7), it is possible that the anticholinergic properties of certain H_1 antagonists are largely responsible for this effect.

Anticholinergic Effects. Many of the first-generation H_1 antagonists tend to inhibit responses to acetylcholine that are mediated by muscarinic receptors. These atropine-like actions are sufficiently prominent in some of the drugs to be manifest during clinical usage (*see* below). The second-generation H_1 antagonists have no effect on muscarinic receptors.

Local Anesthetic Effect. Some H_1 antagonists have local anesthetic activity, and a few are more potent than procaine. *Promethazine* (PHENERGAN) is especially active. However, the concentrations required for this effect are several orders higher than those that antagonize histamine.

Absorption, Fate, and Excretion. The H_1 antagonists are well absorbed from the gastrointestinal tract. Following oral administration, peak plasma concentrations are achieved in 2 to 3 hours and effects usually last 4 to 6 hours; however, some of the drugs are much longer acting (Table 25–1).

Extensive studies of the metabolic fate of the older H_1 antagonists are limited. Diphenhydramine, given orally, reaches a maximal concentration in the blood in about 2 hours, remains at about this level for another 2 hours, and then falls exponentially with a plasma elimination half-time of about 4 to 8 hours. The drug is widely distributed throughout the body, including the CNS. Little, if any, is excreted unchanged in the urine; most appears there as metabolites. Other first-generation H_1 antagonists appear to be eliminated in much the same way (*see* Paton and Webster, 1985).

Information on the concentrations of these drugs achieved in the skin and mucous membranes is lacking. However, significant inhibition of "wheal-and-flare" responses to the intradermal injection of histamine or allergen may persist for 36 hours or more after treatment with some longer-acting H_1 antagonists, even when concentrations of the drugs in plasma are very low. Such results emphasize the need for flexibility in the interpretation of the recommended dosage schedules (*see* Table 25–1); less frequent dosage may suffice. Doxepin, a tricyclic antidepressant (*see* Chapter 19), is one of the most potent antihistamines available; it is about 800 times more potent than diphenhydramine (Sullivan 1982; Richelson, 1979). This may account for the observation that doxepin can be effective in the treatment of chronic urticaria when other antihistamines have failed; it also is available as a topical preparation.

Like many other drugs that are metabolized extensively, H_1 antagonists are eliminated more rapidly by children than by adults and more slowly in those with severe liver disease. H_1-receptor antagonists are among the many drugs that induce hepatic microsomal enzymes, and they may facilitate their own metabolism (*see* Paton and Webster, 1985; Simons and Simons, 1988).

The second-generation H_1 antagonist loratadine is rapidly absorbed from the gastrointestinal tract and

Table 25–1
Preparations and Dosage of Representative H_1-Receptor Antagonists*

CLASS AND NONPROPRIETARY NAME	TRADE NAME	DURATION OF ACTION, hours	PREPARATIONS†	SINGLE DOSE (ADULT)
First-Generation Agents				
Tricyclic Dibenzoxepins				
Doxepin hydrochloride	SINEQUAN	6–24	O, L, T	10–150 mg
Ethanolamines				
Carbinoxamine maleate	RONDEC,¶ others	3–6	O, L	4–8 mg
Clemastine fumarate	TAVIST, others	12	O, L	1.34–2.68 mg
Diphenhydramine hydrochloride	BENADRYL; others	4–6	O, L, I, T	25–50 mg
Dimenhydrinate	DRAMAMINE; others	4–6	O, L, I	50–100 mg
Ethylenediamines				
Pyrilamine maleate	POLY–HISTINE-D¶	4–6	O, L, T	25–50 mg
Tripelennamine hydrochloride	PBZ	4–6	O	25–50 mg, 100 mg (sustained release)
Tripelennamine citrate	PBZ	4–6	L	37.5–75 mg
Alkylamines				
Chlorpheniramine maleate	CHLOR-TRIMETON; others	4–6	O, L, I	4 mg 8–12 mg (sustained release) 5–20 mg (injection)
Brompheniramine maleate	BROMPHEN; others	4–6	O, L, I	4 mg 8–12 mg (sustained release) 5–20 mg (injection)
Piperazines				
Hydroxyzine hydrochloride	ATARAX; others	6–24	O, L, I	25–100 mg
Hydroxyzine pamoate	VISTARIL	6–24	O, L	25–100 mg
Cyclizine hydrochloride	MAREZINE	4–6	O	50 mg
Cyclizine lactate	MAREZINE	4–6	I	50 mg
Meclizine hydrochloride	ANTIVERT; others	12–24	O	12.5–50 mg
Phenothiazines				
Promethazine hydrochloride	PHENERGAN; others	4–6	O, L, I, S	12.5–50 mg
Piperidines				
Cyproheptadine hydrochloride§	PERIACTIN	4–6	O, L	4 mg
Phenindamine tartrate	NOLAHIST	4–6	O	25 mg

Table 25–1 (continued)

CLASS AND NONPROPRIETARY NAME	TRADE NAME	DURATION OF ACTION, hours	PREPARATIONS†	SINGLE DOSE (ADULT)
Second-Generation Agents				
Alkylamines				
Acrivastine‡	SEMPREX-D¶	4–6	O	8 mg
Piperazines				
Cetirizine hydrochloride‡	ZYRTEC	12–24	O	5–10 mg
Phthalazinones				
Azelastine hydrochloride‡	ASTELIN	12–24	T	2 sprays per nostril
Piperidines				
Levocabastine hydrochloride	LIVOSTIN	6	T	One drop
Loratadine	CLARITIN	24	O, L	10 mg
Fexofenadine	ALLEGRA	12	O	60 mg

*For a discussion of phenothiazines, *see* Chapter 20.
†Preparations are designated as follows: O, oral solids; L, oral liquids; I, Injection; S, suppository; T, topical. Many H_1-receptor antagonists also are available in preparations that contain multiple drugs.
‡Has mild sedating effects.
¶Trade name drug also contains other medications.
§Also has antiserotonin properties.

metabolized in the liver to an active metabolite by the hepatic microsomal P450 system (Simons and Simons, 1994). Consequently, metabolism of this drug can be affected by competition for the P450 enzymes by other drugs. Two other second-generation H_1 antagonists that had been marketed previously, astemizole and terfenadine, also underwent P450 metabolism to active metabolites. Both of these drugs were found in rare cases to induce a potentially fatal arrhythmia, *torsades de pointes,* when their metabolism was impaired, such as by liver disease or drugs that inhibit the 3A family of P450 enzymes. This led to the withdrawal of terfenadine and astemizole from the market in 1998 and 1999. Loratadine, cetirizine (the active metabolite of hydroxyzine), fexofenadine (the active metabolite of terfenadine), and azelastine lack the propensity to prolong repolarization and induce *torsades de pointes* (DuBuske, 1999). Cetirizine, loratadine, and fexofenadine are all well absorbed and are excreted mainly in the unmetabolized form. Cetirizine and loratadine are primarily excreted into the urine, whereas fexofenadine is primarily excreted in the feces (Brogden and McTavish, 1991; Spencer *et al.,* 1993; Barnes *et al.,* 1993; Russell *et al.,* 1998).

Side Effects. ***Sedation and Other Common Adverse Effects.*** The side effect with the highest incidence in the first-generation H_1 antagonists, which is not a feature of the second-generation agents, is sedation. Although sedation may be a desirable adjunct in the treatment of some patients, it may interfere with the patient's daytime activities. Concurrent ingestion of alcohol or other CNS depressants produces an additive effect that impairs motor skills (Roehrs *et al.,* 1993). Other untoward reactions referable to central actions include dizziness, tinnitus, lassitude, incoordination, fatigue, blurred vision, diplopia, euphoria, nervousness, insomnia, and tremors.

The next most frequent side effects involve the digestive tract and include loss of appetite, nausea, vomiting, epigastric distress, and constipation or diarrhea. Their incidence may be reduced by giving the drug with meals. H_1 antagonists appear to increase appetite and cause weight gain in rare patients. Other side effects that apparently are caused by the antimuscarinic actions of some of the first-generation H_1-receptor antagonists include dryness of the mouth and respiratory passages, sometimes inducing cough, urinary retention or frequency, and dysuria. These effects are not observed with second-generation H_1 antagonists.

Mutagenicity. Results of one short-term study (Brandes *et al.,* 1994) with an unconventional mouse model indicated that melanoma and fibrosarcoma tumor lines had an increased rate of growth when injected into mice receiving certain H_1 antagonists. However, conventional studies with animals and clinical experience do not suggest carcinogenicity for H_1-receptor antagonists (Food and Drug Administration, 1994).

Other Adverse Effects. Drug allergy may develop when H_1 antagonists are given orally, but more commonly it results from topical application. Allergic dermatitis is not uncommon; other hypersensitivity reactions include drug fever and photosensitization. Hematological complications such as leukopenia, agranulocytosis, and hemolytic anemia are very rare. Teratogenic effects have been noted in response to piperazine compounds, but extensive clinical studies have not demonstrated any association between the use of such H_1 antagonists and fetal anomalies in human beings. Since H_1 antagonists interfere with skin tests for allergy, they must be withdrawn well before such tests are performed.

In acute poisoning with H_1 antagonists, their central excitatory effects constitute the greatest danger. The syndrome includes hallucinations, excitement, ataxia, incoordination, athetosis, and convulsions. Fixed, dilated pupils with a flushed face, together with sinus tachycardia, urinary retention, dry mouth, and fever, lend the syndrome a remarkable similarity to that of atropine poisoning. Terminally, there is deepening coma with cardiorespiratory collapse and death, usually within 2 to 18 hours. Treatment is along general symptomatic and supportive lines.

Available H_1 Antagonists. Below are summarized the therapeutic and side effects of a number of H_1 antagonists, based on their chemical structure. Representative preparations are listed in Table 25–1.

Dibenzoxepin Tricyclics (Doxepin). Doxepin is the only drug in this class. Doxepin is marketed as a tricyclic antidepressant (*see* Chapter 19). However, it also is a remarkably potent H_1 antagonist. It can cause drowsiness and is associated with anticholinergic effects. Doxepin is much better tolerated by patients who have depression than by those who do not. In nondepressed patients, sometimes even very small doses, *e.g.*, 20 mg, may be poorly tolerated because of disorientation and confusion.

Ethanolamines (Prototype: Diphenhydramine). The drugs in this group possess significant antimuscarinic activity and have a pronounced tendency to induce sedation. About half of those who are treated with conventional doses of these drugs experience somnolence. The incidence of gastrointestinal side effects, however, is low with this group.

Ethylenediamines (Prototype: Pyrilamine). These include some of the most specific H_1 antagonists. Although their central effects are relatively feeble, somnolence occurs in a fair proportion of patients. Gastrointestinal side effects are quite common.

Alkylamines (Prototype: Chlorpheniramine). These are among the most potent H_1 antagonists. The drugs are not so prone as some H_1 antagonists to produce drowsiness and are among the more suitable agents for daytime use; but again, a significant proportion of patients do experience sedation. Side effects involving CNS stimulation are more common in this than in other groups.

First-Generation Piperazines. The oldest member of this group, *chlorcyclizine,* has a more prolonged action and produces a comparatively low incidence of drowsiness. *Hydroxyzine* is a long-acting compound that is widely used for skin allergies; its considerable CNS-depressant activity may contribute to its prominent antipruritic action. Cyclizine and meclizine have been used primarily to counter motion sickness, although promethazine and diphenhydramine (dimenhydrinate) are more effective (as is scopolamine; *see* below).

Second-Generation Piperazines (Cetirizine). Cetirizine is the only drug in this class. It has minimal anticholinergic effects. It also has negligible penetration into the brain but is associated with a somewhat higher incidence of drowsiness than the other second-generation H_1 antagonists.

Phenothiazines (Prototype: Promethazine). Most drugs of this class are H_1 antagonists and also possess considerable anticholinergic activity. Promethazine, which has prominent sedative effects, and its many congeners are now used primarily for their antiemetic effects (*see* Chapter 38).

First-Generation Piperidines (Cyproheptadine, Phenindamine). Cyproheptadine is unique in that it has both antihistamine and antiserotonin activity. Cyproheptadine and phenindamine cause drowsiness and also have significant anticholinergic effects.

Second-Generation Piperidines (Prototype: Terfenadine). As mentioned, terfenadine and astemizole were early marketed H_1 antagonists in this class but have since been withdrawn because they induced the potentially fatal arrhythmia, *torsades de pointes*. The drugs currently marketed in this class, which are devoid of this side effect, are loratadine and fexofenadine. These agents are highly selective for H_1 receptors and are devoid of significant anticholinergic actions. These agents also penetrate poorly into the CNS. Taken together, these properties appear to account for the low incidence of side effects of piperidine agents.

Therapeutic Uses

H_1 antagonists have an established and valued place in the symptomatic treatment of various immediate hypersensitivity reactions. In addition, the central properties of some of the series are of therapeutic value for suppressing motion sickness or for sedation.

Diseases of Allergy. H_1 antagonists are most useful in acute types of allergy that present with symptoms of rhinitis, urticaria, and conjunctivitis. Their effect, however, is confined to the suppression of symptoms attributable to the histamine released by the antigen–antibody reaction. In bronchial asthma, histamine antagonists have limited beneficial effects and are not useful as sole therapy (*see* Chapter 28). In the treatment of systemic anaphylaxis, in which autacoids other than histamine play major roles, the mainstay of therapy is epinephrine, with histamine antagonists having only a subordinate and adjuvant role. The same is true for severe angioedema, in which laryngeal swelling constitutes a threat to life.

Other allergies of the respiratory tract are more amenable to therapy with H_1 antagonists. The best results are obtained in seasonal rhinitis and conjunctivitis (hay fever, pollinosis), in which these drugs relieve the sneezing, rhinorrhea, and itching of eyes, nose, and throat. A gratifying response is obtained in most patients, especially at the beginning of the season when pollen counts are low; however, the drugs are less effective when the allergens are in abundance, when exposure to them

is prolonged, and when nasal congestion has become prominent. Topical preparations of antihistamines such as *levocabastine* (LIVOSTIN) have been shown to be effective in allergic conjunctivitis and rhinitis (Janssens and Vanden Bussche, 1991). A topical ophthalmic preparation of this agent is available in the United States (*see* Chapter 66) and nasal sprays are being tested.

Certain of the allergic dermatoses respond favorably to H_1 antagonists. Benefit is most striking in acute urticaria, although the itching in this condition is perhaps better controlled than are the edema and the erythema. Chronic urticaria is less responsive, but some benefit may occur in a fair proportion of patients. Furthermore, the combined use of H_1 and H_2 antagonists is effective for some individuals if therapy with an H_1 antagonist has failed. As mentioned above, doxepin is sometimes effective in the treatment of chronic urticaria that is refractory to other antihistamines. Angioedema also is responsive to treatment with H_1 antagonists, but the paramount importance of epinephrine in the severe attack must be reemphasized, especially in the life-threatening involvement of the larynx (*see* Chapter 10). Here, however, it may be appropriate to administer additionally an H_1 antagonist by the intravenous route. H_1 antagonists also have a place in the treatment of pruritus. Some relief may be obtained in many patients suffering atopic dermatitis and contact dermatitis (although topical corticosteroids are more effective) and in such diverse conditions as insect bites and ivy poisoning. Various other pruritides without an allergic basis sometimes respond to antihistamine therapy, usually when the drugs are applied topically but sometimes when they are given orally. However, the possibility of producing allergic dermatitis with local application of H_1 antagonists must be recognized. Again, doxepin may be more effective in suppressing histamine-mediated symptoms in the skin, in this case pruritus, than are other antihistamines. Since these drugs inhibit allergic dermatoses, they should be withdrawn well before skin testing for allergies.

The urticarial and edematous lesions of serum sickness respond to H_1 antagonists, but fever and arthralgia often do not.

Many drug reactions attributable to allergic phenomena respond to therapy with H_1 antagonists, particularly those characterized by itch, urticaria, and angioedema; reactions of the serum-sickness type also respond to intensive treatment. However, explosive release of histamine generally calls for treatment with epinephrine, with H_1 antagonists being accorded a subsidiary role. Nevertheless, prophylactic treatment with an H_1 antagonist may suffice to reduce symptoms to a tolerable level when a drug known to be a histamine liberator is to be given.

Common Cold. Despite persistent popular belief, H_1 antagonists are without value in combating the common cold. The weak anticholinergic effects of the older agents may tend to lessen rhinorrhea, but this drying effect may do more harm than good, as may their tendency to induce somnolence.

Motion Sickness, Vertigo, and Sedation. Although scopolamine, given orally, parenterally, or transdermally, is the most effective of all drugs for the prophylaxis and treatment of motion sickness, some H_1 antagonists are useful in a broad range of milder conditions and offer the advantage of fewer adverse effects. These drugs include dimenhydrinate and the piperazines (*e.g.*, cyclizine, meclizine). Promethazine, a phenothiazine, is more potent and more effective and its additional antiemetic properties may be of value in reducing vomiting, but its pronounced sedative action usually is disadvantageous. Whenever possible, the various drugs should be administered an hour or so before the anticipated motion. Dosing after the onset of nausea and vomiting rarely is beneficial.

Some H_1 antagonists, notably dimenhydrinate and meclizine, are often of benefit in vestibular disturbances, such as Meniere's disease, and in other types of true vertigo. Only promethazine has usefulness in treating the nausea and vomiting subsequent to chemotherapy or radiation therapy for malignancies; however, other effective antiemetic drugs are available (*see* Chapter 38).

Diphenhydramine can be used to reverse the extrapyramidal side effects caused by phenothiazines. The anticholinergic actions of this agent also can be utilized in the early stages of treatment of patients with Parkinson's disease (*see* Chapter 22), but it is less effective than other agents such as *trihexyphenidyl* (ARTANE).

The tendency of certain of the H_1-receptor antagonists to produce somnolence has led to their use as hypnotics. H_1 antagonists, principally diphenhydramine, often are present in various proprietary remedies for insomnia that are sold over the counter. While these remedies generally are ineffective in the recommended doses, some sensitive individuals may derive benefit. The sedative and mild antianxiety activities of hydroxyzine and diphenhydramine have contributed to their use as weak anxiolytics.

H_3-RECEPTOR–MEDIATED ACTIONS: AGONISTS AND ANTAGONISTS

Originally the H_3 receptor was described as a presynaptic receptor present on histaminergic nerve terminals in the CNS that exerted feedback regulation of histamine synthesis and release (Arrang *et al.*, 1983). Since then, H_3 receptors have been found to function in a wide variety of tissues as feedback inhibitors not only of histamine but also of other neurotransmitters, including acetylcholine, dopamine, norepinephrine, and serotonin (Leurs *et al.*, 1998). Like H_1 and H_2 receptors, H_3 receptors are G protein–coupled receptors; their occupation results in a decrease of Ca^{2+} influx into the cell. *(R)-α-Methylhistamine* is a selective H_3 agonist, being approximately 1500 times more selective for the H_3 receptor than for the H_2 receptor and 3000 times more selective for the H_3 receptor than for the H_1 receptor (Timmerman, 1990). The development of this and other potent, selective agonists of the H_3 receptor has proven invaluable in defining the functions of the H_3 receptor. The H_3 receptor was cloned in 1999 (Lovenberg *et al.*, 1999). This important advance should now allow the development of genetically modified animals to further characterize H_3 receptor-mediated actions. Recently, evidence was obtained for the presence of a second isoform of the H_3 receptor in guinea pig brain (Tardivel-Lacome *et al.*, 2000). Whether there are two isoforms in human beings and whether the two isoforms in the guinea pig exhibit functional differences is unknown.

Many early H_3 antagonists such as impromidine and burimamide had mixed effects, since they also were agonists for the H_2 receptor. *Thioperamide* was the first specific H_3 antagonist available experimentally (Timmerman, 1990). This compound is still the most widely used H_3 antagonist and has potent pharmacological properties (*see* below). Other H_3 antagonists being developed include the competitive inhibitor *clobenpropit* and the irreversible inhibitor *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ).

H_3 receptors are known to function as feedback inhibitors in a wide variety of organ systems. In the CNS, H_3-receptor agonists cause sedation by opposing H_1-induced wakefulness (Monti, 1993). In the gastrointestinal tract, H_3 receptors antagonize H_1-induced ileal contraction as well as downregulate histamine (and thus gastrin) levels through autoregulatory actions in the gastric mucosa (Hollande *et al.*, 1993). The H_1-bronchoconstrictor response is opposed by an H_3-bronchodilatory response in the pulmonary tree.

Ishikawa and Sperelakis (1987) first documented the existence of H_3 receptors in the cardiovascular system. These authors documented that H_3-receptor agonists depressed perivascular sympathetic neurotransmission and caused vasodilation in the guinea pig mesenteric arteries. Subsequently, H_3 receptors were discovered on sympathetic nerve terminals in the human saphenous vein, where H_3-receptor agonists inhibited sympathetic outflow and norepinephrine release (Molderings *et al.*, 1992). In addition to interference with sympathetic vasoconstriction, H_3 receptors also have been shown to have negative chronotrophic effects in the atria. H_3 receptors probably have minimal effects in baseline normal states but may inhibit norepinephrine release during stresses such as ischemia (Imamura *et al.*, 1994).

Currently, much attention is focused on the therapeutic potential of ligands of the H_3 receptor in a variety of pathological situations. Agonists have potential use as gastroprotective, antiinflammatory, and anticonvulsant agents and in the treatment of septic shock, heart failure, and myocardial infarction. Antagonists have potential use in treating obesity, cognitive dysfunction, and attention-deficit–hyperactivity disorder in children (Leurs *et al.*, 2000). A number of potent, selective agonists and antagonists of H_3 receptors have been developed, but none has yet been approved for clinical use.

BRADYKININ AND KALLIDIN AND THEIR ANTAGONISTS

A variety of factors including tissue damage, allergic reactions, viral infections, and other inflammatory events activate a series of proteolytic reactions that generate bradykinin and kallidin in the tissues (*see* Wachtfogel *et al.*, 1993). These peptides are autacoids that act locally to produce pain, vasodilation, increased vascular permeability, and the synthesis of prostaglandins. Thus, they constitute a subset of the large number of mediators that contribute to the inflammatory response.

During the past several years, a number of interesting discoveries have been made concerning kinins and their receptors. Kinin metabolites that were formerly considered inactive degradation products now are considered potent mediators of inflammation and pain. These peptides interact with specific receptors whose presence is induced by tissue injury. Based on this information, novel avenues for therapeutic intervention in chronic inflammatory conditions may be possible.

History. In the 1920s and 1930s, Frey and his associates Kraut and Werle characterized a hypotensive substance in urine and showed that similar material could be obtained from saliva, plasma, and a variety of tissues. Since the pancreas was a rich source, they named this material *kallikrein* after an old Greek synonym for that organ, *kallikréas*. By 1937, Werle, Götze, and Keppler had established that kallikreins generate a pharmacologically active substance from some inactive precursor present in plasma. In 1948, Werle and Berek named the active substance *kallidin* and showed it to be a polypeptide cleaved from a plasma globulin that they termed *kallidinogen* (*see* Werle, 1970).

Interest in the field intensified when Rocha e Silva and associates (1949) reported that trypsin and certain snake venoms acted on plasma globulin to produce a substance that lowered blood pressure and caused a slowly developing contraction of the gut. Because of this slow response, they named this substance *bradykinin,* a term derived from the Greek words *bradys,* meaning "slow," and *kinein,* meaning "to move." In 1960, the nonapeptide bradykinin was isolated by Elliott and coworkers and synthesized by Boissonnas and associates. Shortly thereafter, kallidin was found to be a decapeptide—bradykinin with an additional lysine residue at the amino terminus. These substances are members of a group of polypeptides with related chemical structures and pharmacological properties that are widely distributed in nature. For the whole group, the generic term *kinins* has been adopted, and kallidin and bradykinin are referred to as plasma kinins.

In 1970, Ferreira *et al.* reported the isolation of a bradykinin-potentiating factor from the venom of the Brazilian snake, *Bothrops,* and Ondetti *et al.* (1971) subsequently reported the isolation of angiotensin converting-enzyme (ACE) inhibitors from the same venom. Later, it was shown that ACE and kininase II are the same enzyme (Erdos, 1977). ACE inhibitors (*see* Chapter 31) now are widely used in the treatment of hypertension, diabetic nephropathy, congestive heart failure, and post–myocardial infarction.

In 1980, Regoli and Barabé divided the kinin receptors into B_1 and B_2 classes based on the rank order of potency of kinin analogs. The B_1 and B_2 receptors have now been cloned. The development of first-generation kinin-receptor antagonists occurred in the mid-1980s (Vavrek and Stewart, 1985). Second-generation, receptor-specific kinin antagonists were developed in the early 1990s. These antagonists have led to increasing understanding of the actions of kinins. The development of a B_2-receptor "knockout" mouse (Borkowski *et al.*, 1995) has furthered our understanding of the role of bradykinin in the regulation of cardiovascular homeostasis.

The Endogenous Kallikrein–Kininogen–Kinin System

Synthesis and Metabolism of Kinins. Bradykinin is a nonapeptide (*see* Table 25–2). Kallidin has an additional lysine residue at the amino-terminal position and is sometimes referred to as lysyl-bradykinin. The two peptides are cleaved from α_2 globulins termed *kininogens*. There are two kininogens, high-molecular-weight (HMW) and low-molecular-weight (LMW) kininogen. A number of serine proteases will generate kinins, but the highly specific proteases that release bradykinin

Table 25–2
Structure of Kinin Agonists and Antagonists, Listed from Carboxyl Terminus

NAME	STRUCTURE	FUNCTION
Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Agonist, $B_2 > B_1$
Kallidin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Agonist, $B_2 \simeq B_1$
des-Arg^9-bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	Agonist, B_1
des-Arg^{10}-kallidin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	Agonist, B_1
RMP-7	H-Arg-Pro-Hyp-Gly-Thi-Ser-Pro-4Mc-Tyr(ψCH_2NH)-Arg-OH	Agonist, B_2
des-Arg^9-[Leu^8]-bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu	Antagonist, B_1
HOE 140	[D-Arg]-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Oic-Arg	Antagonist, B_2
CP 0127	B(D-Arg-Arg-Pro-Hyp-Gly-Phe-Cys-D-Phe-Leu-Arg)$_2$	Antagonist, B_2
FRI 73657	Nonpeptide	Antagonist, B_2

Hyp, *trans*-4-hydroxy-Pro; Thi, β-(2-thienyl)-Ala; Tic, [D]-1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl; Oic, (3as,7as)-octahydroindol-2-yl-carbonyl. B, bissuccimidohexane.

and kallidin from the kininogens are termed *kallikreins* (*see* Figure 25–4 and below).

Kallikreins. Bradykinin and kallidin are cleaved from high- and low-molecular-weight kininogens by plasma or tissue kallikrein, respectively. Plasma kallikrein and tissue kallikrein are distinct enzymes, and they are activated by different mechanisms (Bhoola *et al.*, 1992). Plasma prekallikrein is an inactive protein of about 88,000 daltons that is bound in a 1:1 complex with its substrate, HMW kininogen. The cascade is restrained by the protease inhibitors present in plasma. Among the most important are the inhibitor of the activated first component of complement (C1-INH) and α_2-macroglobulin. Under experimental conditions, the kallikrein-kinin system is activated by the binding of factor XII, also known as Hageman factor, to negatively charged surfaces. Factor XII, a protease that is common to both the kinin and the intrinsic coagulation cascades (*see* Chapter 55), undergoes autoactivation and, in turn, activates kallikrein. Importantly, kallikrein further activates factor XIIa, thereby exerting a positive feedback on the system (*see* Proud and Kaplan, 1988). *In vivo,* factor XII does not undergo autoactivation upon binding to endothelial cells. Instead, the binding of a HMW kininogen/prekallikrein complex to a multiprotein receptor complex leads to activation of prekallikrein by a membrane-associated, cysteine protease. Kallikrein activates factor XII, cleaves HMW kininogen, and activates prourokinase (Schmaier *et al.,* 1999; Colman, 1999).

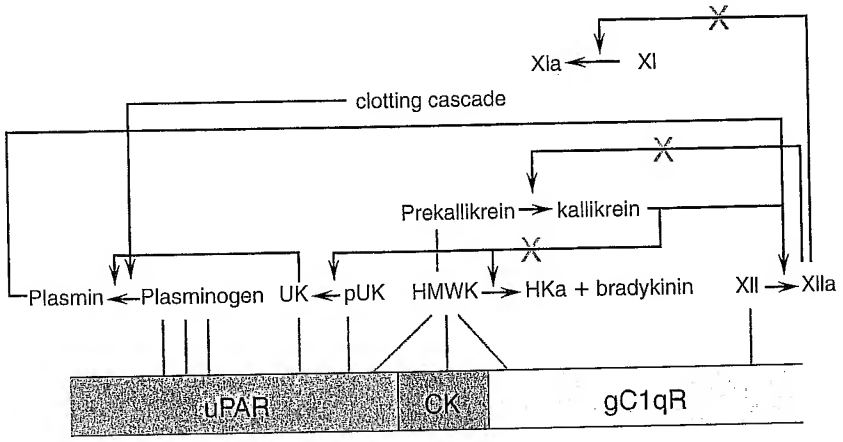


Figure 25–4. Schematic diagram of kinin production on the endothelial cell surface.

The high-molecular-weight kininogen (HMWK)–prekallikrein complex binds to a multiprotein complex, comprising the globular C1q receptor (gC1qR), cytokeratin 1 (CK), and the urokinase receptor (uPAR), on the surface of endothelial cells. This leads to activation of prekallikrein by a membrane-associated cysteine protease (not shown). Kallikrein then cleaves its substrate, HMWK, liberating kinin-free kininogen (HKa) and bradykinin from the surface. Note the relationship between kinin formation and the coagulation and fibrinolytic systems. Clotting factors are indicated by Roman numerals. Blue X's indicate the sites of inhibition by C1 esterase inhibitor (C1-INH). pUK indicates prourokinase; UK indicates urokinase. (Modified from Colman, 1999, with permission.)

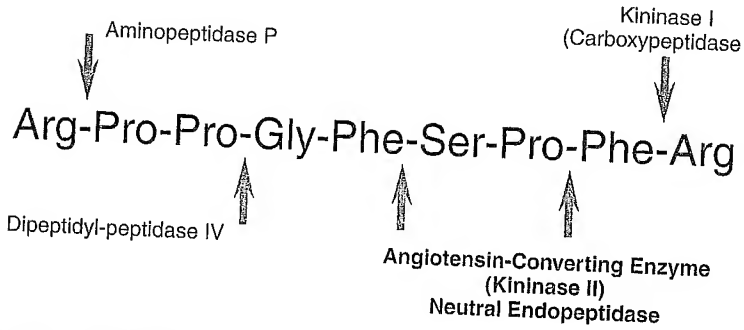


Figure 25–5. Schematic diagram of the degradation of bradykinin.

Bradykinin and kallidin are inactivated primarily by kininase II or angiotensin converting enzyme (ACE). Neutral endopeptidase also cleaves bradykinin and kallidin at the carboxyl terminus. In addition, aminopeptidase P inactivates bradykinin by hydrolyzing the N-terminal Arg^1-Pro^2 bond, leaving bradykinin susceptible to further degradation by dipeptidyl-peptidase IV. Bradykinin and kallidin are converted to their respective des-Arg^9 metabolites by kininase I. Unlike the parent compounds, these kinin metabolites are potent ligands for B_1-kinin receptors but not B_2-kinin receptors.

The human tissue kallikrein family includes three members: true tissue kallikrein (hKLK1), prostate-specific antigen (PSA, hKLK3), and a PSA-like proteinase (hKLK2). Only true tissue kallikrein exhibits kininogenase activity. Compared to plasma kallikrein, tissue kallikrein is a smaller protein (molecular mass of 29,000 daltons). It is synthesized as a preproprotein in the epithelial cells or secretory cells of a number of tissues including salivary glands, pancreas, prostate, and distal nephron. Tissue kallikrein is also expressed in human neutrophils. It acts locally near its site of origin (Fukushima *et al.*, 1985; Evans *et al.*, 1988). The synthesis of tissue prokallikrein is regulated by a number of factors, including aldosterone in the kidney and salivary gland and androgens in certain other glands. The secretion of the tissue prokallikrein also may be regulated; for example, its secretion from the pancreas is enhanced by stimulation of the vagus nerve (*see* Proud and Kaplan, 1988; Margolius, 1989). The activation of tissue prokallikrein to kallikrein requires proteolytic cleavage. In human beings, the sequence of these activation events is not well delineated (Bhoola *et al.*, 1992).

Kininogens. The two substrates for the kallikreins, HMW and LMW kininogen, are products of a single gene that arise by alternative processing of mRNA. HMW and LMW kininogen have been divided into functional domains. The HMW kininogen contains 626 amino acid residues; the internal bradykinin sequence of 9 amino acid residues, domain 4, connects an amino-terminal "heavy chain" sequence (362 amino acids) containing domains 1 through 3 and a carboxyl-terminal "light chain" sequence (255 amino acids) containing domains D5H and D6. LMW kininogen is identical to the larger form of the protein from the amino terminus through the bradykinin sequence; its short light chain differs (Takagaki *et al.*, 1985). HMW kininogen is cleaved by plasma and tissue kallikrein to yield bradykinin and kallidin, respectively. LMW kininogen is a substrate only for the tissue kallikrein and the product is kallidin (*see* Nakanishi, 1987). In addition to serving as precursors of bradykinin and kallidin, the kininogens inhibit cysteine proteinase, inhibit thrombin binding, and exhibit antiadhesive and profibrinolytic properties.

Metabolism. The decapeptide kallidin is about as active as the nonapeptide bradykinin and need not be converted to the latter to exert its characteristic effects. Some conversion of kallidin to bradykinin occurs as the amino-terminal lysine residue is removed by a plasma aminopeptidase. However, this reaction is slow relative to the rate of inactivation by hydrolysis at the carboxyl terminus. The minimal effective structure required to elicit the classical responses is that of the nonapeptide (Figure 25–5).

The kinins have an evanescent existence—their half-life in plasma is only about 15 seconds. Moreover, in a single passage through the pulmonary vascular bed some 80% to 90% of the kinins may be destroyed (*see* Ryan, 1982). Plasma concentrations of bradykinin have been difficult to define because of its short half-life. Inadequate inhibition of kininogenases or kininases in the blood can lead to artifactual formation or degradation of bradykinin during blood collection. For this reason, physiological concentrations of bradykinin have been reported to range from picomolar to femtomolar (Pellacani *et al.*, 1992).

The principal catabolizing enzyme in the lung and in other vascular beds is the dipeptidyl carboxypeptidase kininase II, known in another context as angiotensin converting enzyme (*see* Chapter 31). Removal of the carboxyl-terminal dipeptide abolishes kinin-like activity. Neutral endopeptidase also inactivates kinins by removing the carboxyl-terminal dipeptide. A slower-acting enzyme, arginine carboxypeptidase (carboxypeptidase-N; kininase I), removes the carboxyl-terminal arginine residue producing des-Arg^9-bradykinin and des-Arg^{10}-kallidin (Table 25–2), which are themselves potent B_1-kinin receptor agonists (Burch and Kyle, 1992; Trifilieff *et al.*, 1993). A familial carboxypeptidase-N deficiency has been described in which affected individuals with low levels of this enzyme display angioedema or urticaria (*see* below) (Mathews *et al.*, 1980). Finally, aminopeptidase-P inactivates bradykinin by cleaving the amino-terminus arginine, rendering bradykinin susceptible to further cleavage by dipeptidyl peptidase IV.

Bradykinin Receptors. There are at least two distinct receptors for kinins, which have been designated B_1 and B_2 (Regoli and Barabé, 1980). The classical bradykinin receptor,

now designated the B_2 receptor, selectively binds bradykinin and kallidin (*see* Table 25–2) and is constitutively present in most normal tissues. B_2 receptors mediate the majority of the effects of bradykinin and kallidin in the absence of inflammation. The B_1 receptor selectively binds to the carboxy-terminal des-Arg metabolites of bradykinin and kallidin (*see* Table 25–2) and is less prevalent than the B_2 receptor in most tissues. B_1 receptors are present in normal vascular smooth muscle. B_1 receptors are upregulated by inflammation and by cytokines, endotoxins, and growth factors (Regoli and Barabé, 1980; Dray and Perkins, 1993). During physiological insults such as trauma, tissue damage, or inflammation, B_1 receptor effects may predominate. The signaling mechanisms of B_1 receptors are less well characterized than are those of B_2 receptors.

The B_2 receptor is a G protein–coupled 7-transmembrane-domain receptor that activates phospholipase A_2 and phospholipase C, apparently *via* interaction with distinct G proteins. Kinin-induced phospholipase C activation through a $G_{\alpha q}$ complex leads to an increase in IP_3 (and thus cytosolic Ca^{2+}, with subsequent enhanced nitric oxide synthesis and release) and diacylglycol (and thus protein kinase C activity). Bradykinin has been shown to activate Ca^{2+}-dependent, Ca^{2+}-independent, and atypical isoforms of protein kinase C (Tippmer *et al.*, 1994). The stimulation of phospholipase A_2 *via* $G_{\alpha i}$ liberates arachidonic acid from membrane-bound phospholipids (Schrör, 1992). The liberated arachidonic acid then can be metabolized to a variety of potent inflammatory mediators and the vasodilator prostacycin (*see* Chapter 26). Binding of bradykinin to the B_2 receptor leads to internalization of the agonist-receptor complex, and to desensitization.

Based on the inability of B_1 and B_2 antagonists to compete for specific bradykinin binding in guinea pig trachea, the existence of a B_3 receptor has been suggested (Farmer *et al.*, 1989; Farmer and DeSiato, 1994). In addition, the presence of B_4 and B_5 receptors on opossum esophageal smooth muscle cells has been suggested. However, studies with more potent kinin antagonists have not supported the existence of the B_3, B_4, or B_5 receptors. These studies indicate that the guinea pig bronchoconstriction proposed as a B_3-receptor effect actually may represent previously unappreciated functions of the B_2 receptor (Regoli *et al.*, 1993).

Functions and Pharmacology of Kallikreins and Kinins

The availability of newer and more specific bradykinin antagonists and the generation of bradykinin-receptor "knockout" mice have led to significant advances in our understanding of the roles of the kinins. Of current interest is the role of these compounds in diverse areas such as pain, inflammation and chronic inflammatory diseases, the cardiovascular system, and reproduction.

Pain. The kinins are powerful algesic agents that cause an intense, burning pain when applied to the exposed base of a blister. Bradykinin excites primary sensory neurons and provokes the release of neuropeptides such as substance P, neurokinin A, and calcitonin gene–related peptide (Geppetti, 1993). In acute pain, B_2 receptors mediate bradykinin algesia. This pain is significantly reduced by B_2 antagonists but not by B_1 antagonists. The pain of chronic inflammation appears to involve increased numbers of B_1 receptors.

Inflammation. Injected kinins mimic inflammation. Measurement of the components of the kinin cascade and the effects of bradykinin antagonists indicates that kinins participate in a variety of inflammatory diseases. Plasma kinins increase permeability in the microcirculation. The effect, like that of histamine and serotonin in some species, is exerted on the small venules and involves separation of the junctions between endothelial cells. This, together with an increased hydrostatic pressure gradient, causes edema. Such edema, coupled with stimulation of nerve endings (*see* below), results in a "wheal-and-flare" response to intradermal injections in human beings.

Bradykinin is formed, and there is depletion of the components of the kinin cascade during episodes of swelling, laryngeal edema, and abdominal pain in hereditary angioedema (Proud and Kaplan, 1988). B_1 receptors on inflammatory cells such as macrophages can elicit production of the inflammatory mediators IL-1 and tumor necrosis factor α (TNF-α) (Dray and Perkins, 1993). Increased levels of kinins have been shown to be present in a number of chronic inflammatory diseases. These include rhinitis caused by inhalation of antigens and that associated with rhinoviral infection. Kinins also may play significant roles in conditions such as gout, disseminated intravascular coagulation, inflammatory bowel disease, rheumatoid arthritis, and asthma. The kinins also may contribute to the changes in the bones seen in chronic inflammatory states. Kinins stimulate bone resorption through B_1 and possibly B_2 receptors, perhaps by osteoblast-mediated osteoclast activation (Lerner, 1994).

Respiratory Disease. The kinins have been implicated in the pathophysiology of allergic airway disorders such as asthma and rhinitis. Inhalation or intravenous injection of kinins causes bronchospasm in asthmatic patients but not in normal individuals. Similarly, nasal challenge with bradykinin induces sneezing and serious glandular secretions in patients with allergic rhinitis. Bradykinin-induced bronchoconstriction is blocked by anticholinergic agents but not by antihistamines or cyclooxygenase inhibitors. A bradykinin B_2-receptor antagonist also has been shown to improve pulmonary function in patients with severe asthma. Repeated inhalation of bradykinin results in an attenuated response, decreasing the bronchoconstriction

in response to bradykinin as well as that in response to adenosine 5′ monophosphate (Polosa *et al.,* 1992).

Cardiovascular System. The kallikrein-kinin system was first implicated in the regulation of blood pressure in the 1920s and 1930s when Frey and Werke identified kallikrein as a hypotensive substance in urine. Since then, numerous investigators have reported that urinary kallikrein concentrations are decreased in individuals with high blood pressure. In experimental animals and human beings, infusion of bradykinin causes vasodilation and lowers blood pressure. Bradykinin causes vasodilation through B_2-receptor-dependent effects on endothelial nitric oxide, prostacyclin, and the poorly characterized endothelium-derived hyperpolarizing factor (Vanhoutte, 1989).

The availability of specific bradykinin antagonists and genetically altered animals has greatly enhanced our understanding of the role of endogenous bradykinin in the regulation of blood pressure (Madeddu, 1993; Madeddu *et al.,* 1997). Basal blood pressure is normal in B_2-receptor antagonist–treated or B_2-receptor knockout animals. However, these animals exhibit an exaggerated blood-pressure response to salt loading or activation of the renin–angiotensin system. These data suggest that the endogenous kallikrein–kinin system plays a minor role in the regulation of blood pressure under normal circumstances, but it may play an important role in hypertensive states.

In addition to causing vasodilation, the kallikrein–kinin system appears to exert a number of cardioprotective effects. Bradykinin contributes to the protective effect of preconditioning the heart against ischemia and reperfusion injury (Linz and Schölkens, 1992). In the presence of endothelial cells, bradykinin prevents vascular smooth muscle cell growth and proliferation. Bradykinin stimulates tPA release from the vascular endothelium and may inhibit thrombin (Brown *et al.,* 1999; Hasan *et al.,* 1996). Through these mechanisms, bradykinin may contribute to the endogenous defense against cardiovascular events such as myocardial infarction and stroke.

Kinins also may increase sympathetic outflow *via* central and peripheral nervous mechanisms (Dominiak *et al.,* 1992; Schwieler and Hjemdahl, 1992; Madeddu, 1993). These findings suggest that kinins may mediate hypertension in some circumstances *via* the sympathetic nervous system, though this remains speculative.

Kidney. Renal kinins act as paracrine hormones to regulate urine volume and composition (Saitoh *et al.,* 1995). Kallikrein is synthesized and secreted by the connecting cells of the distal nephron. Tissue kininogen and kinin receptors are present in the cells of the collecting duct. Like other vasodilators, kinins increase renal blood flow. Bradykinin also causes natriuresis by inhibiting sodium reabsorption at the cortical collecting duct. Renal kallikreins are increased by treatment with mineralocorticoids, ACE inhibitors, and neutral endopeptidase inhibitors.

Other Effects. The rat uterus is especially sensitive to contraction by kinins through the B_2 receptor. Kinins also function in the male reproductive system in areas such as spermatogenesis and in promoting sperm motility, possibly through a B_2 receptor on the sperm membrane (Schill and Miska, 1992). Kinins promote dilation of the fetal pulmonary artery, closure of the ductus arteriosus, and constriction of the umbilical vessels, all of which occur in the adjustment from fetal to neonatal circulation.

The kallikrein–kinin system also functions in a wide variety of other areas in the body, serving to mediate edema formation and smooth muscle contraction. The bradykinin-induced, slowly developing contraction of the isolated guinea pig ileum first prompted the name bradykinin. The kinins also have neurochemical effects in the CNS, in addition to their ability to disrupt the blood–brain barrier and allow increased CNS penetration (*see* Inamura *et al.,* 1994).

Potential Therapeutic Uses. Bradykinin contributes to many of the effects of the widely used cardiovascular drugs, the ACE inhibitors. Aprotinin, a nonspecific kallikrein antagonist, is administered to patients undergoing coronary bypass in order to minimize bleeding and blood transfusion requirements. Kinin agonists have potential value in increasing the delivery of chemotherapeutic agents beyond the blood–brain barrier. Based on the physiology outlined above, kinin antagonists are being tested in a number of inflammatory conditions.

Kallikrein Inhibitors. *Aprotinin* (TRASYLOL) is a natural proteinase inhibitor obtained from bovine lung. Aprotinin inhibits many of the mediators of the inflammatory response, fibrinolysis, and thrombin generation following cardiopulmonary bypass surgery, including kallikrein and plasmin. In several placebo-controlled, double-blind studies, administration of aprotinin during bypass reduced requirements for blood products in patients undergoing coronary artery bypass grafting (Levy *et al.,* 1995). Aprotinin is given as a loading dose followed by a continuous infusion during surgery. Hypersensitivity reactions, including anaphylactic or anaphylactoid reactions, may occur with aprotinin. The rate of such reactions is 2.7% in patients who have been previously exposed to aprotinin and higher in patients who have been exposed to aprotinin within the last six months. A test dose of aprotinin is recommended prior to full dosing. Aprotinin can interfere with an activated clotting time, used to determine the

effectiveness of heparin anticoagulation. For this reason, alternate methods must be used to determine the degree of anticoagulation in patients treated with aprotinin during cardiopulmonary bypass. In one multicenter study of aprotinin, there was an increased closure rate of saphenous vein grafts in patients treated with aprotinin compared to those treated with placebo; there were no differences in rates of myocardial infarction or death.

Angiotensin Converting Enzyme Inhibitors. The ACE inhibitors are widely used in the treatment of hypertension and have been shown to reduce mortality in patients with diabetic nephropathy, left ventricular dysfunction, previous myocardial infarction, and coronary artery disease. ACE inhibitors block the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor and growth promoter (*see* Chapter 31). Data from studies using the specific bradykinin B_2 antagonist HOE 140 demonstrate that bradykinin also contributes to many of the protective effects of ACE inhibitors. For example, in animal models, administration of HOE 140 attenuates the favorable effects of ACE inhibitors on blood pressure, on myocardial infarct size, and on ischemic preconditioning (Linz and Schölkens, 1992). Bradykinin receptor antagonism also attenuates the blood pressure–lowering effects of acute ACE inhibition in human beings (Gainer *et al.,* 1998). The contribution of bradykinin to the effects of ACE inhibitors may result not only from decreased degradation of bradykinin but also from enhanced receptor sensitivity (Marcic *et al.,* 1999).

Occasional patients receiving ACE inhibitors have experienced angioedema. This can occur at any time, but often occurs shortly after initiating therapy. This is an effect of ACE inhibitors as a group and is thought to be due to inhibition of kinin metabolism by ACE (Slater *et al.,* 1988). ACE-inhibitor-associated angioedema is more common in blacks than in Caucasians. Severe anaphylactoid reactions can occur in patients taking ACE inhibitors who are undergoing dialysis with polyacrylonitrile AN69 membranes (Schulman *et al.,* 1993; Verresen *et al.,* 1994). In these patients, kinins are produced by activation of factor XII by the negatively charged surface of the polyacrylonitrile AN69 membrane while ACE inhibition diminishes the clearance of these kinins. A more common side effect of ACE inhibitors (especially in women) is a chronic nonproductive cough that dissipates upon cessation of the ACE inhibitor. The finding that angiotensin AT_1-receptor-subtype antagonists do not cause cough has been taken as presumptive evidence for the role of bradykinin in ACE inhibitor–induced cough.

Preliminary data suggest that bradykinin also may contribute to the effects of the AT_1-receptor antagonists. During AT_1-receptor blockade, angiotensin II concentrations increase. Renal bradykinin concentrations also increase through effects of angiotensin II on the unopposed AT_2 subtype receptor (Carey *et al.,* 2000). Whether or not bradykinin contributes to the clinical effects of the AT_1-receptor antagonists remains to be determined. In addition, a new class of antihypertensive agents, the combined ACE/neutral endopeptidase inhibitors, is undergoing testing. To the extent that these drugs inhibit two kinin-degrading enzymes, bradykinin may be expected to contribute significantly to their clinical effects.

Bradykinin Antagonists. The introduction of a D-aromatic amino acid in place of the proline residue at position seven conferred antagonist activity to bradykinin and blocked the action of angiotensin converting enzyme. The addition of an N-terminal D-arginine residue also increased the half-life of these antagonists by blocking the action of aminopeptidase P. Nevertheless, the early kinin antagonists were partial agonists and had short half-lives due to enzymatic degradation by carboxypeptidase N *in vivo*. In the early 1990s, a longer-acting, more selective kinin antagonist, HOE 140, was developed by substituting synthetic amino acids at position seven [D-tetrahydroisoquinoline-3-carboxylic acid (Tic)] and position eight [octahydroindole-2-carboxylic acid (Oic)]. The substitution of the Oic residue at position eight blocked degradation by carboxypeptidase P. The availability of HOE 140 has contributed dramatically to our understanding of the role of bradykinin in human health and disease.

CP-0127, a 6-Cys substituted, cross-linked analog of bradykinin, has been tested in the treatment of sepsis in humans in a randomized prospective trial (Fein *et al.,* 1997). In a study of 504 patients with systemic inflammatory response syndrome (SIRS) and presumed sepsis, there was no effect of the bradykinin analog on 28-day survival. However, there was an improvement in risk-adjusted survival in a predefined subset of patients with gram-negative sepsis. A small pilot study in patients with edema following head trauma suggests that bradykinin-receptor antagonism may reduce intracranial pressure.

The development of orally active, nonpeptide antagonists promises to make bradykinin antagonism therapeutically feasible in the treatment of disease. The first of these, WIN64338, suffered from having muscarinic cholinergic activity. More recently, the nonpeptide antagonist FR173657 has been shown to decrease bradykinin-induced edema and hypotension in animal models.

Bradykinin Agonists. RMP-7 [H-Arg-Pro-Hyp-Gly-Thi-Ser-Pro-4Me-Tyr(ΨCH_2NH)-Arg-OH] is a bradykinin analog that has been rendered resistant to degradation by bradykinin-metabolizing enzymes by the introduction of a reduced peptide bond at the carboxyl terminus. RMP-7 increases the permeability of the blood–brain barrier, and clinical trials are evaluating its efficacy in enhancing the delivery of chemotherapeutic agents

into the CNS of patients with primary brain tumors (Cloughesy *et al.,* 1993).

PROSPECTUS

The refinement of the structure–function relationships among histamine receptor subtypes has allowed the continued development of H_2-selective antagonists for the treatment of peptic ulcers (*see* Chapter 37). The further understanding of the physiological and pathophysiological roles of H_3-receptor subtypes in the CNS and elsewhere similarly may permit the development of new and more selective therapeutic tools.

The availability of new peptide and nonpeptide bradykinin antagonists provides the tools for further elucidation of the role of the kallikrein–kinin system in health and disease. Ongoing clinical trials will determine the efficacy of bradykinin agonists in enhancing the delivery of chemotherapeutic agents across the blood–brain barrier. Clinical trials will better define the contribution of bradykinin to the cardioprotective effects of ACE inhibitors, AT_1-receptor antagonists, and combined ACE/neutral endopeptidase inhibitors.

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QUANTITATION OF DRUG–RECEPTOR INTERACTIONS AND ELICITED EFFECT

Receptor Pharmacology

The major aim of receptor pharmacology is to understand and quantify the effects of chemicals (drugs) on biological systems. This is important in the therapeutic arena because drugs are nearly always used therapeutically in systems different from those in which they were discovered and tested. Biological systems interpret the effects of drugs in different ways, and these interpretations can be confusing. What is needed is a standard scale of drug activity that transcends biological systems and can be used to predict the effects of the drug in all systems. Receptor pharmacology strives to furnish the tools to accomplish this goal.

The basic currency of receptor pharmacology is the dose–response curve, a depiction of the observed effect of a drug as a function of its concentration in the receptor compartment. Figure 2–4*A* shows a typical dose–response curve; it reaches a maximal asymptote value when the drug occupies all of the receptor sites. The range of concentrations needed to fully depict the dose–response relationship usually is too wide to be useful in the format shown in Figure 2–4*A*. Most dose–response curves are therefore plotted with the logarithm of the concentration as the *x* axis (see Figure 2–4*B*). Dose–response curves have three basic properties: threshold, slope, and maximal asymptote; these parameters characterize and quantitate the activity of the drug.

In general, drugs can do two things to receptors: (1) bind to them and (2) possibly change their behavior toward the host cell system. The first function is governed by the chemical property of *affinity*, ruled by the chemical forces that cause the drug to associate with the receptor. The second is governed by a quantity referred to as *efficacy*. Efficacy is the information encoded in a drug's chemical structure that causes the receptor to change accordingly when the drug is bound. Historically, efficacy has been treated operationally as a proportionality constant that quantifies the extent of functional change imparted to a receptor upon binding a drug.

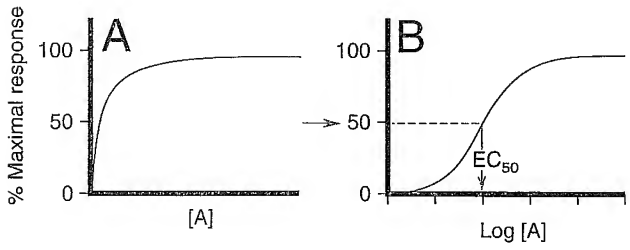

*Figure 2–4. Graded responses (*y *axis as a percent of maximal response) expressed as a function of the concentration of drug A present at the receptor.*

The hyperbolic shape of the curve in panel A becomes sigmoid when plotted semilogarithmically, as in panel B. The concentration of drug that produces 50 percent of the maximal response quantifies drug activity and is referred to as the EC_{50} (effective concentration for 50 percent response).

Classical Receptor Theory. Receptor occupancy theory, in which it is assumed that response emanates from a receptor occupied by a drug, has its basis in the law of mass action, with modifying constants added to accommodate experimental findings. Agonism was described by modification of this model by Ariëns (1954), Stephenson (1956), and Furchgott (1966). Stephenson introduced another important concept, *stimulus*, which is the initial effect of drug upon the receptor itself; stimulus is then processed by the system to yield the observable response. Antagonism was modeled by Gaddum (1937, 1957) and Schild (1957) to determine the affinity of antagonists.

The basic components of drug receptor–mediated response are shown in Figure 2–5. Affinity is measured by the equilibrium

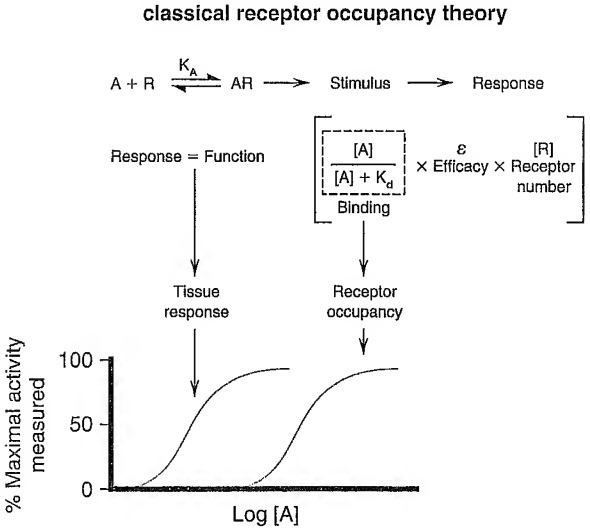

Figure 2–5. Classical receptor occupancy theory.

Drug A binds to receptor R to form a complex AR, the proximal signal from which is then processed by the cell to produce an observable response. Occupancy of the receptor is given by the Langmuir adsorption isotherm: $[A]/([A] + K_d)$. The amplitude of the signal for each bound receptor is determined by the efficacy ε, which is multiplied by receptor concentration $[R]$ to yield the total receptor-mediated stimulus. A cascade of biochemical events in the cell processes this stimulus to produce the response. Fractional receptor binding and fractional final response are shown as functions of drug concentration, $[A]$.

dissociation constant of the drug–receptor complex (denoted K_d); the fraction of receptors occupied by the drug is determined by the concentration of drug and K_d, as shown (*see* Figure 2–5). Intrinsic efficacy is a proportionality constant (denoted ε) that defines the power of the drug to induce response. The product of occupancy, intrinsic efficacy, and receptor number yields the total receptor-mediated stimulus given to the system. Stimulus is conveyed to physiological effectors by biochemical reactions to produce the response. It should be noted that efficacy is a function of occupancy and the stimulus–response function (comprising all of the biochemical reactions that take place to translate agonist binding into response) and amplifies stimulus. Therefore, the location of the dose–response curves for response is shifted to the left of the receptor occupancy curve (Figure 2–5).

Before discussion of the quantitation of drug–receptor effects, it is worth considering this amplification process further because it can control the observed response to a drug.

Transmission of Receptor Stimulus by the Target Tissue. The activation of a receptor by a drug can be thought of as an initial signal that is then amplified by the cell. Different cells have different amplification properties; thus a weak receptor signal may produce no visible response in one cell type and a powerful signal in another. The amplification properties of the cell (referred to as the *stimulus-response capability*) control the observed outcome of drug–receptor interaction, as shown in Figure 2–6 for three hypothetical drugs and three different cell types. In cell I, which amplifies a stimulus relatively

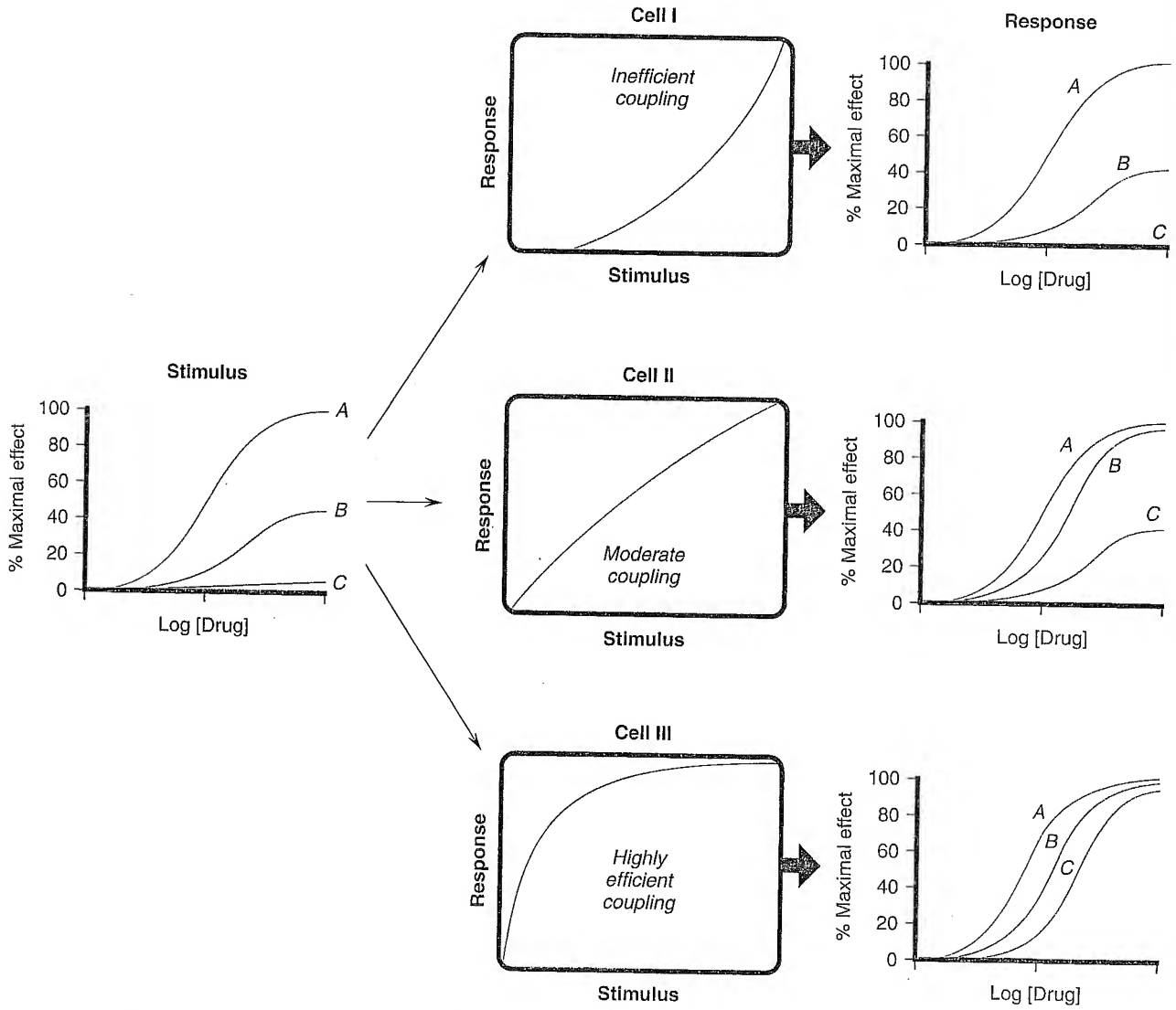

Figure 2–6. Different efficiencies of cellular stimulus-response processing can produce different levels of response for three agonists of differing efficacies.

See text for details.

weakly, drug A produces a full tissue response and would be labeled a full agonist. Drug B produces a partial (submaximal) tissue response and would be a partial agonist. Drug C produces no response, but nevertheless occupies the receptor and therefore would antagonize the effects of either drug A or drug B; it would be referred to as an antagonist. When these same drugs are tested on cell II, which has a more efficiently coupled stimulus-response mechanism, drug A remains a full agonist, drug B is now also a full agonist, and drug C, which had insufficient efficacy to cause a physiological response in cell I, is now a partial agonist. The properties of these drugs have not changed; only the efficiency of the signaling system has changed. Thus, the labels for these drugs change as well. Drug B goes from a partial agonist to a full agonist and drug C goes from an antagonist to a partial agonist. This progression continues when these drugs are tested in cell III, which has even more efficient signaling machinery. Now all three drugs act as full agonists (Figure 2–6). This example illustrates the potential fallacy of classifying drugs on the basis of what they do rather than what they are. What drugs do depends on the receptor and its associated signaling proteins; classification by magnitude of physiological effect can be seriously misleading when drugs are tested in one cellular format for therapeutic use in another. The alternative is to classify drugs according to the magnitude of their two molecular properties: affinity for the receptor and efficacy once bound. By quantifying these system-independent properties, drug activity can be predicted in all systems as long as the identity of the receptor is known.

Quantifying Agonism. Drugs have two observable properties in biological systems: potency and magnitude of effect (when a biological response is produced). Potency is controlled by four factors: two relate to the biological system containing the receptors (receptor density and efficiency of the stimulus–response mechanisms of the tissue) and two relate to the interaction of drug with its receptor (affinity and efficacy). When the relative potency of two agonists of equal efficacy is measured in the same biological system, downstream signaling effects cancel and the comparison yields a relative measure of the affinity and efficacy of the two agonists (*see* Figure 2–7*A*). Thus, measuring agonist potency ratios is one method of measuring the capability of different agonists to induce a response in a test system and for predicting comparable activity in another. Another method of estimating agonist activity is to compare maximal asymptotes in systems where the agonists do not produce system maximal response (Figure 2–7*B*). The advantage of using maxima is that this property is solely dependent upon efficacy, whereas potency is a mixed function of both affinity and efficacy.

Quantifying Antagonism. Characteristic patterns of antagonism are associated with certain mechanisms of blockade of receptors. One is simple competitive antagonism, whereby a drug that lacks intrinsic efficacy but retains affinity competes with the agonist for the binding site. The characteristic pattern of such antagonism is the concentration-dependent production of a parallel shift to the right of the agonist dose–response curve with no change in the maximal asymptotic response (Figure 2–8*A*). The magnitude of the rightward shift of the curve depends only upon the concentration of the antagonist and its affinity for the receptor. The affinity of a competitive antagonist for its receptor can therefore be determined according to its concentration-dependent ability to shift the dose–response curve for an agonist rightward, as first noted by Schild (1957). Note that a partial agonist similarly can compete with a "full" agonist for binding to the receptor. However, increasing concentrations of a partial agonist will inhibit response to a finite level characteristic of the drug's intrinsic efficacy; a competitive antagonist will reduce the response to zero. Partial agonists thus can be used therapeutically to buffer a response by inhibiting untoward stimulation without totally abolishing the stimulus from the receptor.

An antagonist may dissociate so slowly from the receptor as to be essentially irreversible in its action. Under these circumstances, the maximal response to the agonist will be depressed at some antagonist concentrations (Figure 2–8*B*). Operationally, this is referred to as *noncompetitive antagonism,* although the molecular mechanism of action really cannot be unequivocally inferred from the effect. An irreversible antagonist competing for the same binding site as the agonist also can produce the pattern of antagonism shown in Figure 2–8*B*.

Noncompetitive antagonism can be produced by another type of drug, referred to as an *allosteric antagonist.* This type of drug produces its effect by binding a site on the receptor distinct from that of the primary agonist and thereby changing the affinity of the receptor for the agonist (*see* Figure 2–8). In the case of an allosteric antagonist, the affinity of the receptor for the agonist is decreased by the antagonist (*see* Figure 2–8*C*). In

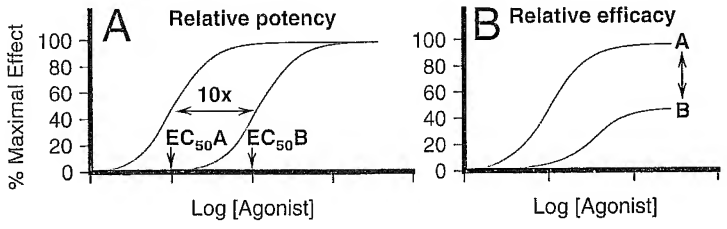

Figure 2–7. Two ways of quantifying agonism.

A. The relative potency of two agonists, when obtained in the same tissue, is a function of their relative affinities and intrinsic efficacies. *B.* In systems where the two drugs do not both produce the maximal response characteristic of the tissue, the observed maximal response is a nonlinear function of their relative intrinsic efficacies.

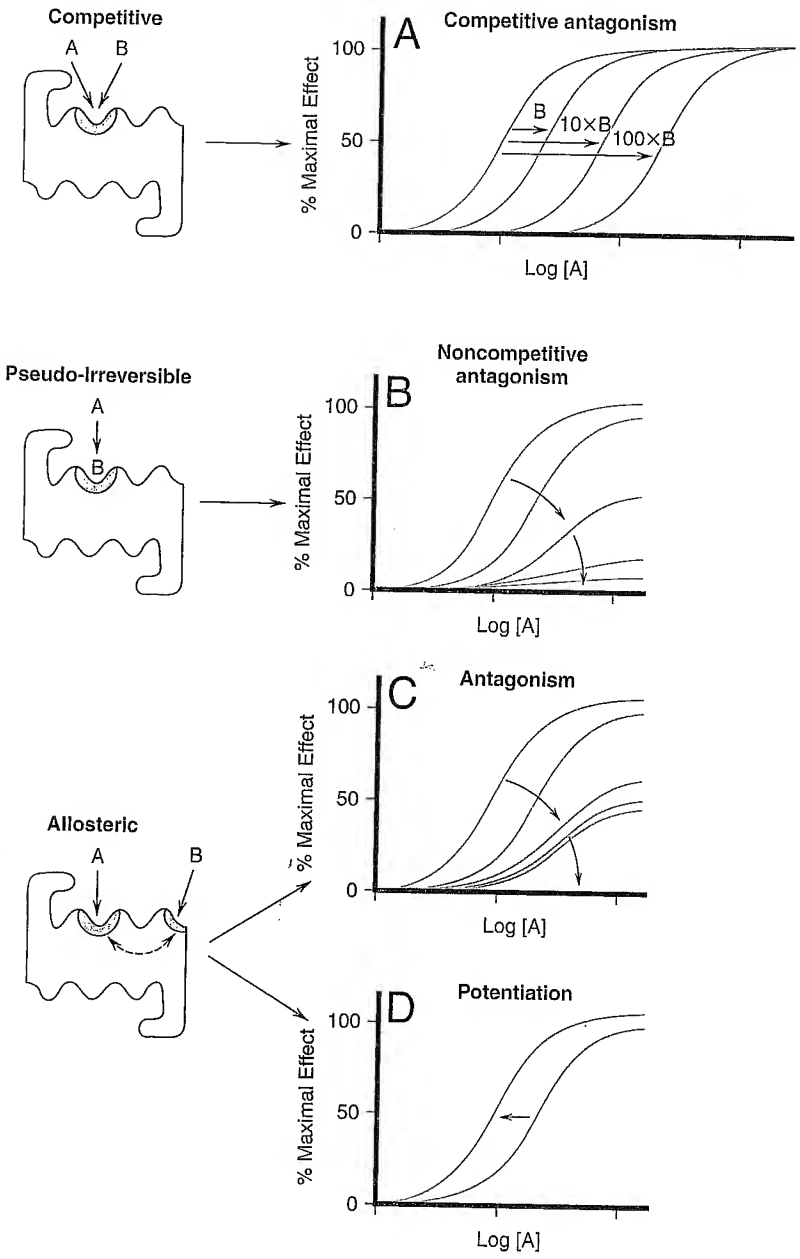

Figure 2–8. Mechanisms of receptor antagonism.

A. Competitive antagonism occurs when the agonist A and antagonist B compete for the same binding site on the receptor. Response curves for the agonist are shifted to the right in a concentration-related manner by the antagonist such that the EC_{50} for the agonist increases linearly with the concentration of the antagonist. ***B.*** If the antagonist binds to the same site as the agonist but does so irreversibly or pseudoirreversibly (slow dissociation but no covalent bond), it causes a shift of the dose–response curve to the right, with further depression of the maximal response. Allosteric effects occur when the ligand B binds to a different site on the receptor to either inhibit response (see panel ***C***) or potentiate response (see panel ***D***). This effect is saturable; inhibition reaches a limiting value when the allosteric site is fully occupied.

contrast, some allosteric effects potentiate the effects of agonists (Figure 2–8*D*). Thus, in cases where the pathology may involve a failing agonist system (*i.e.,* myasthenia gravis, Alzheimer's disease), an allosteric potentiator of the endogenous response would strengthen the signal and, importantly, preserve the natural pattern of response.

By allowing both the overexpression of wild-type receptors and the creation (and discovery) of constitutively active mutant receptors, molecular genetic technology has facilitated the study of a novel class of functional antagonists, the inverse agonists. As discussed above, receptors spontaneously can adopt active conformations that produce a cellular response. The fraction of unoccupied receptors in the active conformation usually is too low to allow observation of their agonist-independent activity; but this activity can be observed readily, either when the receptor is expressed at heterologously high levels or when mutation shifts the conformational equilibrium toward the active form. In these situations, the tissue behaves as if there were an agonist present, and a conventional competitive antagonist has no effect. However, because inverse agonists selectively bind to the inactive form of the receptor and shift the conformational equilibrium toward the inactive state, these agents are capable

of inhibiting agonist-independent or constitutive signaling. In systems that are not constitutively active, inverse agonists will behave exactly like competitive antagonists, which in part explains why the properties of inverse agonists and the number of such agents previously described as competitive antagonists were not appreciated until recently.

It is not known to what extent constitutive receptor activity is a pathologically important phenomenon, and it is therefore unclear to what extent inverse agonism is a therapeutically relevant property. In some cases, however, the preferability of an inverse agonist over a competitive antagonist is obvious. For example, the human herpesvirus KSHV encodes a constitutively active chemokine receptor that generates a second messenger that drives cell growth and viral replication (Arvanitakis *et al.*, 1997). Clearly, in such a case a conventional antagonist would not be useful as the chemokine agonist is not involved, and an inverse agonist would be the only viable intervention.

PROSPECTUS

The continuing identification and expansion of molecular families for receptors, especially at the advent of the human genome era, coupled with the enormous potential for generating new molecules with combinatorial chemistry or recombinant DNA strategies, forecast a new era of diversity and specificity in therapeutic intervention.

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CHAPTER 1

PHARMACOKINETICS

The Dynamics of Drug Absorption, Distribution, and Elimination

Grant R. Wilkinson

To produce its characteristic effects, a drug must be present in appropriate concentrations at its sites of action. Although obviously a function of the amount of drug administered, the concentrations of active, unbound (free) drug attained also depend upon the extent and rate of its absorption, distribution (which mainly reflects relative binding to plasma and tissue proteins), metabolism (biotransformation), and excretion. These disposition factors are depicted in Figure 1–1 and are described in this chapter.

PHYSICOCHEMICAL FACTORS IN TRANSFER OF DRUGS ACROSS MEMBRANES

The absorption, distribution, metabolism, and excretion of a drug all involve its passage across cell membranes. Mechanisms by which drugs cross membranes and the physicochemical properties of molecules and membranes that influence this transfer are, therefore, important. The determining characteristics of a drug are its molecular size and shape, degree of ionization, relative lipid solubility of its ionized and nonionized forms, and its binding to tissue proteins.

When a drug permeates a cell, it obviously must traverse the cellular plasma membrane. Other barriers to drug movement may be a single layer of cells (intestinal epithelium) or several layers of cells (skin). Despite such structural differences, the diffusion and transport of drugs across these various boundaries have many common characteristics, since drugs in general pass through cells rather than between them. The plasma membrane thus represents the common barrier.

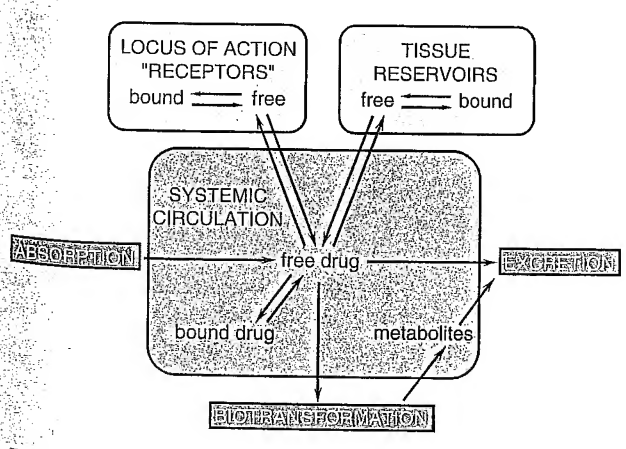


Figure 1–1. Schematic representation of the interrelationship of the absorption, distribution, binding, metabolism, and excretion of a drug and its concentration at its locus of action.

Possible distribution and binding of metabolites are not depicted.

Cell Membranes. The plasma membrane consists of a bilayer of amphipathic lipids, with their hydrocarbon chains oriented inward to form a continuous hydrophobic phase and their hydrophilic heads oriented outward. Individual lipid molecules in the bilayer vary according to the particular membrane and can move laterally, endowing the membrane with fluidity, flexibility, high electrical resistance, and relative impermeability to highly polar molecules. Membrane proteins embedded in the bilayer serve as receptors, ion channels, or transporters to elicit electrical or chemical signaling pathways and provide selective targets for drug actions.

Most cell membranes are relatively permeable to water either by diffusion or by flow resulting from hydrostatic or osmotic differences across the membrane, and bulk flow of water can carry with it drug molecules. Such transport is the major mechanism by which drugs pass across most capillary endothelial membranes. However, proteins and drug molecules bound to them are too large and polar for this type of transport to occur; thus, transcapillary movement is limited to unbound drug. Paracellular transport through intercellular gaps is sufficiently large that passage across most capillaries is limited by blood flow and not by other factors (*see* below). As described later, this type of transport is an important factor in filtration across

glomerular membranes in the kidney. Important exceptions exist in such capillary diffusion, however, since "tight" intercellular junctions are present in specific tissues and paracellular transport in them is limited. Capillaries of the central nervous system (CNS) and a variety of epithelial tissues have tight junctions (*see* below). Although bulk flow of water can carry with it small, water-soluble substances, if the molecular mass of these compounds is greater than 100 to 200 daltons, such transport is limited. Accordingly, most large lipophilic drugs must pass through the cell membrane itself by one or more processes.

Passive Membrane Transport. Drugs cross membranes either by passive processes or by mechanisms involving the active participation of components of the membrane. In the former, the drug molecule usually penetrates by passive diffusion along a concentration gradient by virtue of its solubility in the lipid bilayer. Such transfer is directly proportional to the magnitude of the concentration gradient across the membrane, the lipid:water partition coefficient of the drug, and the cell surface area. The greater the partition coefficient, the higher is the concentration of drug in the membrane and the faster is its diffusion. After a steady state is attained, the concentration of the unbound drug is the same on both sides of the membrane if the drug is a nonelectrolyte. For ionic compounds, the steady-state concentrations will be dependent on differences in pH across the membrane, which may influence the state of ionization of the molecule on each side of the membrane and on the electrochemical gradient for the ion.

Weak Electrolytes and Influence of pH. Most drugs are weak acids or bases that are present in solution as both the nonionized and ionized species. The nonionized molecules are usually lipid-soluble and can diffuse across the cell membrane. In contrast, the ionized molecules are usually unable to penetrate the lipid membrane because of their low lipid solubility.

Therefore, the transmembrane distribution of a weak electrolyte usually is determined by its pK_a and the pH gradient across the membrane. The pK_a is the pH at which half of the drug (weak electrolyte) is in its ionized form. To illustrate the effect of pH on distribution of drugs, the partitioning of a weak acid ($pK_a = 4.4$) between plasma (pH = 7.4) and gastric juice (pH = 1.4) is depicted in Figure 1–2. It is assumed that the gastric mucosal membrane behaves as a simple lipid barrier that is permeable only to the lipid-soluble, nonionized form of the acid. The ratio of nonionized to ionized drug at each pH is readily calculated from the Henderson–Hasselbalch equation. Thus, in plasma, the ratio of nonionized to ionized drug is 1:1000; in gastric juice, the ratio is 1:0.001. These values are given in brackets in Figure 1–2. The total concentration ratio between the plasma and the gastric juice would therefore be 1000:1 if such a system came to a steady state. For a weak base with a pK_a of 4.4, the ratio would be reversed, as would the thick horizontal arrows in Figure 1–2, which indicate the predominant species at

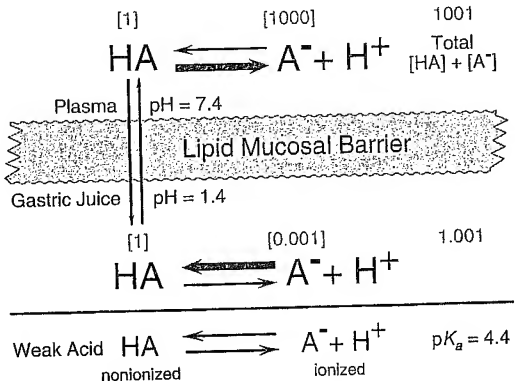


Figure 1–2. Influence of pH on the distribution of a weak acid between plasma and gastric juice, separated by a lipid barrier.

each pH. Accordingly, at steady state, an acidic drug wil accumulate on the more basic side of the membrane anc a basic drug on the more acidic side—a phenomenor termed *ion trapping*. These considerations have obviou implications for the absorption and excretion of drugs, a discussed more specifically below. The establishment o concentration gradients of weak electrolytes across mem branes with a pH gradient is a purely physical proces and does not require an active transport system. All tha is necessary is a membrane preferentially permeable t one form of the weak electrolyte and a pH gradient acros the membrane. The establishment of the pH gradient i however, an active process.

Carrier-Mediated Membrane Transport. While passive di fusion through the bilayer is dominant in the disposition most drugs, carrier-mediated mechanisms also can play an in portant role. *Active transport* is characterized by a requireme for energy, movement against an electrochemical gradient, sa urability, selectivity, and competitive inhibition by cotransporte compounds. The term *facilitated diffusion* describes a carrie mediated transport process in which there is no input of ener and therefore enhanced movement of the involved substan is down an electrochemical gradient. Such mechanisms, whi may be highly selective for a specific conformational structu of a drug, are involved in the transport of endogenous co pounds whose rate of transport by passive diffusion otherwi would be too slow. In other cases, they function as a barr system to protect cells from potentially toxic substances.

The responsible transporter proteins often are express within cell membranes in a domain-specific fashion such t they mediate either drug uptake or efflux, and often such arrangement facilitates vectorial transport across cells. Thus, the liver, a number of basolaterally localized transporters w different substrate specificities are involved in the uptake bile acids and amphipathic organic anions and cations into hepatocyte, and a similar variety of ATP-dependent transport in the canalicular membrane export such compounds into bile. Analogous situations also are present in intestinal and re tubular membranes. An important efflux transporter presen

these sites and also in the capillary endothelium of brain capillaries is P-glycoprotein, which is encoded by the multidrug resistance-1 (*MDR1*) gene, important in resistance to cancer chemotherapeutic agents (Chapter 52). P-glycoprotein localized in the enterocyte also limits the oral absorption of transported drugs since it exports the compound back into the intestinal tract subsequent to its absorption by passive diffusion.

DRUG ABSORPTION, BIOAVAILABILITY, AND ROUTES OF ADMINISTRATION

Absorption describes the rate at which a drug leaves its site of administration and the extent to which this occurs. However, the clinician is concerned primarily with a parameter designated as *bioavailability*, rather than absorption. *Bioavailability* is a term used to indicate the fractional extent to which a dose of drug reaches its site of action or a biological fluid from which the drug has access to its site of action. For example, a drug given orally must be absorbed first from the stomach and intestine, but this may be limited by the characteristics of the dosage form and/or the drug's physicochemical properties. In addition, drug then passes through the liver, where metabolism and/or biliary excretion may occur before it reaches the systemic circulation. Accordingly, a fraction of the administered and absorbed dose of drug will be inactivated or diverted before it can reach the general circulation and be distributed to its sites of action. If the metabolic or excretory capacity of the liver for the agent in question is large, bioavailability will be substantially reduced (the so-called *first-pass effect*). This decrease in availability is a function of the anatomical site from which absorption takes place; other anatomical, physiological, and pathological factors can influence bioavailability (*see* below), and the choice of the route of drug administration must be based on an understanding of these conditions.

Oral (Enteral) *versus* Parenteral Administration. Often there is a choice of the route by which a therapeutic agent may be given, and a knowledge of the advantages and disadvantages of the different routes of administration is then of primary importance. Some characteristics of the major routes employed for systemic drug effect are compared in Table 1–1.

Oral ingestion is the most common method of drug administration. It also is the safest, most convenient, and most economical. Disadvantages to the oral route include limited absorption of some drugs because of their physical characteristics (*e.g.*, water solubility), emesis as a result of irritation to the gastrointestinal mucosa, destruction of some drugs by digestive enzymes or low gastric pH, irregularities in absorption or propulsion in the presence of food or other drugs, and necessity for cooperation on the part of the patient. In addition, drugs in the gastrointestinal tract may be metabolized by the enzymes of the intestinal flora, mucosa, or the liver before they gain access to the general circulation.

The parenteral injection of drugs has certain distinct advantages over oral administration. In some instances, parenteral administration is essential for the drug to be delivered in its active form. Availability is usually more rapid, extensive, and predictable than when a drug is given by mouth. The effective dose therefore can be more accurately delivered. In emergency therapy and when a patient is unconscious, uncooperative, or unable to retain anything given by mouth, parenteral therapy may be a necessity. The injection of drugs, however, has its disadvantages: asepsis must be maintained; pain may accompany the injection; it is sometimes difficult for patients to perform the injections themselves if self-medication is necessary; and there is the risk of inadvertent administration of a drug when it is not intended. Expense is another consideration.

Oral Ingestion. Absorption from the gastrointestinal tract is governed by factors such as surface area for absorption, blood flow to the site of absorption, the physical state of the drug (solution, suspension, or solid dosage form), its water solubility, and concentration at the site of absorption. For drugs given in solid form, the rate of dissolution may be the limiting factor in their absorption, especially if they have low water solubility. Since most drug absorption from the gastrointestinal tract occurs *via* passive processes, absorption is favored when the drug is in the nonionized and more lipophilic form. Based on the pH-partition concept presented in Figure 1–2, it would be predicted that drugs that are weak acids would be better absorbed from the stomach (pH 1 to 2) than from the upper intestine (pH 3 to 6), and *vice versa* for weak bases. However, the epithelium of the stomach is lined with a thick mucous layer, and its surface area is small; by contrast, the villi of the upper intestine provide an extremely large surface area (~200 m^2). Accordingly, the rate of absorption of a drug from the intestine will be greater than that from the stomach even if the drug is predominantly ionized in the intestine and largely nonionized in the stomach. Thus, any factor that accelerates gastric emptying will be likely to increase the rate of drug absorption, while any factor that delays gastric emptying will probably have the opposite effect, regardless of the characteristics of the drug.

Drugs that are destroyed by gastric juice or that cause gastric irritation sometimes are administered in dosage forms with a coating that prevents dissolution in the acidic gastric contents. However, some enteric-coated

Table 1–1
Some Characteristics of Common Routes of Drug Administration*

ROUTE	ABSORPTION PATTERN	SPECIAL UTILITY	LIMITATION AND PRECAUTIONS
Intravenous	Absorption circumvented Potentially immediate effects	Valuable for emergency use Permits titration of dosage Usually required for high-molecular-weight protein and peptide drugs Suitable for large volumes and for irritating substances, when diluted	Increased risk of adverse effects Must inject solutions *slowly,* as a rule Not suitable for oily solutions or insoluble substances
Subcutaneous	Prompt, from aqueous solution Slow and sustained, from repository preparations	Suitable for some insoluble suspensions and for implantation of solid pellets	Not suitable for large volumes Possible pain or necrosis from irritating substances
Intramuscular	Prompt, from aqueous solution Slow and sustained, from repository preparations	Suitable for moderate volumes, oily vehicles, and some irritating substances	Precluded during anticoagulant medication May interfere with interpretation of certain diagnostic tests (*e.g.,* creatine kinase)
Oral ingestion	Variable; depends upon many factors (*see* text)	Most convenient and economical; usually more safe	Requires patient cooperation Availability potentially erratic and incomplete for drugs that are poorly soluble, slowly absorbed, unstable, or extensively metabolized by the liver and/or gut

*See text for more complete discussion and for other routes.

preparations of a drug also may resist dissolution in the intestine, and very little of the drug may be absorbed.

Controlled-Release Preparations. The rate of absorption of a drug administered as a tablet or other solid oral-dosage form is partly dependent upon its rate of dissolution in the gastrointestinal fluids. This factor is the basis for the so-called *controlled-release, extended-release, sustained-release,* or *prolonged-action* pharmaceutical preparations that are designed to produce slow, uniform absorption of the drug for 8 hours or longer. Potential advantages of such preparations are reduction in the frequency of administration of the drug as compared with conventional dosage forms (possibly with improved compliance by the patient), maintenance of a therapeutic effect overnight, and decreased incidence and/or intensity of undesired effects by elimination of the peaks in drug concentration that often occur after administration of immediate-release dosage forms.

Many controlled-release preparations fulfill these expectations. However, such products have some drawbacks. Generally, interpatient variability, in terms of the systemic concentration of the drug that is achieved, is greater for controlled-release than for immediate-release dosage forms. During repeated drug administration, trough drug concentrations resulting from controlled-release dosage forms may not be different from those observed with immediate-release preparations, although the time interval between trough concentrations is greater for a well-designed controlled-release product. It is possible that the dosage form may fail, and "dose-dumping" with resultant toxicity can occur, since the total dose of drug ingested at one time may be several times the amount contained in the conventional preparation. Controlled-release dosage forms are most appropriate for drugs with short half-lives (less than 4 hours). So-called controlled-release dosage forms are sometimes developed for drugs with long half-lives (greater than 12 hours). These usually more expensive products should not be prescribed unless specific advantages have been demonstrated.

Sublingual Administration. Absorption from the oral mucosa has special significance for certain drugs, despite the fact that the surface area available is small. For example, nitroglycerin is effective when retained sublingually because it is nonionic and has a very high lipid solubility. Thus, the drug is absorbed

very rapidly. Nitroglycerin also is very potent; relatively few molecules need to be absorbed to produce the therapeutic effect. Since venous drainage from the mouth is to the superior vena cava, the drug also is protected from rapid hepatic first-pass metabolism, which is sufficient to prevent the appearance of any active nitroglycerin in the systemic circulation if the sublingual tablet is swallowed.

Rectal Administration. The rectal route often is useful when oral ingestion is precluded because the patient is unconscious or when vomiting is present—a situation particularly relevant to young children. Approximately 50% of the drug that is absorbed from the rectum will bypass the liver; the potential for hepatic first-pass metabolism is thus less than that for an oral dose. However, rectal absorption often is irregular and incomplete, and many drugs cause irritation of the rectal mucosa.

Parenteral Injection. The major routes of parenteral administration are intravenous, subcutaneous, and intramuscular. Absorption from subcutaneous and intramuscular sites occurs by simple diffusion along the gradient from drug depot to plasma. The rate is limited by the area of the absorbing capillary membranes and by the solubility of the substance in the interstitial fluid. Relatively large aqueous channels in the endothelial membrane account for the indiscriminate diffusion of molecules regardless of their lipid solubility. Larger molecules, such as proteins, slowly gain access to the circulation by way of lymphatic channels.

Drugs administered into the systemic circulation by any route, excluding the intraarterial route, are subject to possible first-pass elimination in the lung prior to distribution to the rest of the body. The lungs serve as a temporary storage site for a number of agents, especially drugs that are weak bases and are predominantly nonionized at the blood pH, apparently by their partition into lipid. The lungs also serve as a filter for particulate matter that may be given intravenously, and, of course, they provide a route of elimination for volatile substances.

Intravenous. Factors relevant to absorption are circumvented by intravenous injection of drugs in aqueous solution, because bioavailability is complete and rapid. Also, drug delivery is controlled and achieved with an accuracy and immediacy not possible by any other procedure. In some instances, as in the induction of surgical anesthesia, the dose of a drug is not predetermined but is adjusted to the response of the patient. Also, certain irritating solutions can be given only in this manner, since the blood vessel walls are relatively insensitive, and the drug, if injected slowly, is greatly diluted by the blood.

As there are advantages to the use of this route of administration, so are there liabilities. Unfavorable reactions are likely to occur, since high concentrations of drug may be attained rapidly in both plasma and tissues. Because of this, it is advisable to intravenously administer a drug slowly by infusion rather than by rapid injection, and with close monitoring of the patient's response. Furthermore, once the drug is injected there is no retreat. Repeated intravenous injections are dependent upon the ability to maintain a patent vein. Drugs in an oily vehicle or those that precipitate blood constituents or hemolyze erythrocytes should not be given by this route.

Subcutaneous. Injection of a drug into a subcutaneous site often is used. It can be used only for drugs that are not irritating to tissue; otherwise, severe pain, necrosis, and tissue sloughing may occur. The rate of absorption following subcutaneous injection of a drug often is sufficiently constant and slow to provide a sustained effect. Moreover, it may be varied intentionally. For example, the rate of absorption of a suspension of insoluble insulin is slow compared with that of a soluble preparation of the hormone. The incorporation of a vasoconstrictor agent in a solution of a drug to be injected subcutaneously also retards absorption. Absorption of drugs implanted under the skin in a solid pellet form occurs slowly over a period of weeks or months; some hormones are effectively administered in this manner.

Intramuscular. Drugs in aqueous solution are absorbed quite rapidly after intramuscular injection, depending upon the rate of blood flow to the injection site. This may be modulated to some extent by local heating, massage, or exercise. For example, jogging may cause a precipitous drop in blood sugar when insulin is injected into the thigh, rather than into the arm or abdominal wall, since running markedly increases blood flow to the leg. Generally, the rate of absorption following injection of an aqueous preparation into the deltoid or vastus lateralis is faster than when the injection is made into the gluteus maximus. The rate is particularly slower for females after injection into the gluteus maximus. This has been attributed to the different distribution of subcutaneous fat in males and females, since fat is relatively poorly perfused. Very obese or emaciated patients may exhibit unusual patterns of absorption following intramuscular or subcutaneous injection. Very slow, constant absorption from the intramuscular site results if the drug is injected in solution in oil or suspended in various other repository vehicles. Antibiotics often are administered in this manner. Substances too irritating to be injected subcutaneously sometimes may be given intramuscularly.

Intraarterial. Occasionally a drug is injected directly into an artery to localize its effect in a particular tissue or organ—for example, in the treatment of liver tumors and head/neck cancers. Diagnostic agents are sometimes administered by this route. Intraarterial injection requires great care and should be reserved

for experts. The first-pass and cleansing effects of the lung are not available when drugs are given by this route.

Intrathecal. The blood-brain barrier and the blood–cerebrospinal fluid barrier often preclude or slow the entrance of drugs into the CNS. Therefore, when local and rapid effects of drugs on the meninges or cerebrospinal axis are desired, as in spinal anesthesia or acute CNS infections, drugs are sometimes injected directly into the spinal subarachnoid space. Brain tumors also may be treated by direct intraventricular drug administration.

Pulmonary Absorption. Provided that they do not cause irritation, gaseous and volatile drugs may be inhaled and absorbed through the pulmonary epithelium and mucous membranes of the respiratory tract. Access to the circulation is rapid by this route, because the lung's surface area is large. The principles governing absorption and excretion of anesthetic and other therapeutic gases are discussed in Chapters 13, 14, and 16.

In addition, solutions of drugs can be atomized and the fine droplets in air (aerosol) inhaled. Advantages are the almost instantaneous absorption of a drug into the blood, avoidance of hepatic first-pass loss, and, in the case of pulmonary disease, local application of the drug at the desired site of action. For example, drugs can be given in this manner for the treatment of bronchial asthma (*see* Chapter 28). Past disadvantages, such as poor ability to regulate the dose and cumbersomeness of the methods of administration, have to a large extent been overcome by technological advances, including metered-dose inhalers and more reliable aerolizers.

Pulmonary absorption is an important route of entry of certain drugs of abuse and of toxic environmental substances of varied composition and physical states. Both local and systemic reactions to allergens may occur subsequent to inhalation.

Topical Application. ***Mucous Membranes.*** Drugs are applied to the mucous membranes of the conjunctiva, nasopharynx, oropharynx, vagina, colon, urethra, and urinary bladder primarily for their local effects. Occasionally, as in the application of synthetic antidiuretic hormone to the nasal mucosa, systemic absorption is the goal. Absorption through mucous membranes occurs readily. In fact, local anesthetics applied for local effect sometimes may be absorbed so rapidly that they produce systemic toxicity.

Skin. Few drugs readily penetrate the intact skin. Absorption of those that do is dependent on the surface area over which they are applied and to their lipid solubility, since the epidermis behaves as a lipid barrier (*see* Chapter 65). The dermis, however, is freely permeable to many solutes; consequently, systemic absorption of drugs occurs much more readily through abraded, burned, or denuded skin. Inflammation and other conditions that increase cutaneous blood flow also enhance absorption. Toxic effects sometimes are produced by absorption through the skin of highly lipid-soluble substances (*e.g.*, a lipid-soluble insecticide in an organic solvent). Absorption through the skin can be enhanced by suspending the drug in an oily vehicle and rubbing the resulting preparation into the skin. Because hydrated skin is more permeable than dry skin, the dosage form may be modified or an occlusive dressing may be used to facilitate absorption. Controlled-release topical patches are becoming increasingly available. A patch containing scopolamine, placed behind the ear where body temperature and blood flow enhance absorption, releases sufficient drug to the systemic circulation to protect the wearer from motion sickness. Transdermal estrogen replacement therapy yields low maintenance levels of estradiol while minimizing the high estrone metabolite levels observed following oral administration.

Eye. Topically applied ophthalmic drugs are used primarily for their local effects (*see* Chapter 66). Systemic absorption that results from drainage through the nasolacrimal canal is usually undesirable. In addition, drug that is absorbed after such drainage is not subject to first-pass hepatic elimination. Unwanted systemic pharmacological effects may occur for this reason when β-adrenergic receptor antagonists are administered as ophthalmic drops. Local effects usually require absorption of the drug through the cornea; corneal infection or trauma thus may result in more rapid absorption. Ophthalmic delivery systems that provide prolonged duration of action (*e.g.*, suspensions and ointments) are useful additions to ophthalmic therapy. Ocular inserts, developed more recently, provide continuous delivery of low amounts of drug. Very little is lost through drainage; hence, systemic side effects are minimized.

Bioequivalence. Drugs are not administered as such; instead, they are formulated into drug dosage forms. Drug products are considered to be pharmaceutical equivalents if they contain the same active ingredients and are identical in strength or concentration, dosage form, and route of administration. Two pharmaceutically equivalent drug products are considered to be bioequivalent when the rates and extents of bioavailability of the active ingredient in the two products are not significantly different under suitable test conditions. In the past, dosage forms of a drug from different manufacturers and even different lots of preparations from a single manufacturer sometimes differed in their bioavailability. Such differences were seen primarily among oral dosage forms of poorly soluble, slowly absorbed drugs. They result from differences in crystal form, particle size, or other physical characteristics of the drug that are not rigidly controlled in formulation and manufacture of the preparations. These factors affect disintegration of the dosage form and dissolution of the drug and hence the rate and extent of drug absorption.

The potential nonequivalence of different drug preparations has been a matter of concern. Strengthened regulatory requirements have resulted in few, if any, documented cases of nonequivalence between approved drug products. The significance of possible nonequivalence of drug preparations is further discussed in connection with drug nomenclature and the choice of drug name in writing prescription orders (*see* Appendix I).

DISTRIBUTION OF DRUGS

Following absorption or administration into the systemic blood, a drug distributes into interstitial and intracellular fluids. This process reflects a number of physiological factors and the particular physicochemical properties of the individual drug. Cardiac output, regional blood flow, and tissue volume determine the rate of delivery and potential amount of drug distributed into tissues. Initially, liver, kidney, brain, and other well-perfused organs receive most of the drug, whereas delivery to muscle, most viscera, skin,

and fat is slower. This second distribution phase may require minutes to several hours before the concentration of drug in tissue is in distribution equilibrium with that in blood. The second phase also involves a far larger fraction of body mass than does the initial phase and generally accounts for most of the extravascularly distributed drug. With exceptions such as the brain, diffusion of drug into the interstitial fluid occurs rapidly because of the highly permeable nature of the capillary endothelial membrane. Thus, tissue distribution is determined by the partitioning of drug between blood and the particular tissue. Lipid solubility is an important determinant of such uptake as is any pH gradient between intracellular and extracellular fluids for drugs that are either weak acids or bases. However, in general, ion trapping associated with the latter factor is not large, since the pH difference (7.0 *versus* 7.4) is small. The more important determinant of blood:tissue partitioning is the relative binding of drug to plasma proteins and tissue macromolecules.

Plasma Proteins. Many drugs are bound to plasma proteins, mostly to plasma albumin for acidic drugs and to α_1-acid glycoprotein for basic drugs; binding to other plasma proteins generally occurs to a much smaller extent. The binding is usually reversible; covalent binding of reactive drugs such as alkylating agents occurs occasionally.

The fraction of total drug in plasma that is bound is determined by the drug concentration, its affinity for the binding sites, and the number of binding sites. Simple mass-action relationships determine the unbound and bound concentrations (*see* Chapter 2). At low concentrations of drug (less than the plasma-protein binding dissociation constant), the fraction bound is a function of the concentration of binding sites and the dissociation constant. At high drug concentrations (greater than the dissociation constant), the fraction bound is a function of the number of binding sites and the drug concentration. Therefore, plasma binding is a saturable and nonlinear process. For most drugs, however, the therapeutic range of plasma concentrations is limited; thus, the extent of binding and the unbound fraction is relatively constant. The percentage values listed in Appendix II refer only to this situation unless otherwise indicated. The extent of plasma binding also may be affected by disease-related factors. For example, hypoalbuminemia secondary to severe liver disease or the nephrotic syndrome results in reduced binding and an increase in the unbound fraction. Also, conditions resulting in the acute phase reaction response (cancer, arthritis, myocardial infarction, Crohn's disease) lead to elevated levels of α_1-acid glycoprotein and enhanced binding of basic drugs.

Because binding of drugs to plasma proteins is rather nonselective, many drugs with similar physicochemical characteristics can compete with each other and with endogenous substances for these binding sites. For example, displacement of unconjugated bilirubin from binding to albumin by the sulfonamides and other organic anions is known to increase the risk of bilirubin encephalopathy in the newborn. Concern for drug toxicities based on a similar competition between drugs for binding sites has, in the past, been overemphasized. Since drug responses, both efficacious and toxic, are a function of unbound concentrations, steady-state unbound concentrations will change only when either drug input (dosing rate) or clearance of unbound drug is changed [*see* Equation (1–1) and discussion later in this chapter]. Thus, steady-state unbound concentrations are independent of the extent of protein binding. However, for narrow-therapeutic-index drugs, a transient change in unbound concentrations occurring immediately following the dose of a displacing drug could be of concern. A more common problem resulting from competition of drugs for plasma-protein binding sites is misinterpretation of measured concentrations of drugs in plasma, since most assays do not distinguish free drug from bound drug.

Importantly, binding of a drug to plasma proteins limits its concentration in tissues and at its locus of action, since only unbound drug is in equilibrium across membranes. Accordingly, after distribution equilibrium is achieved, the concentration of active, unbound drug in intracellular water is the same as that in plasma except when carrier-mediated transport is involved. Binding also limits glomerular filtration of the drug, since this process does not immediately change the concentration of free drug in the plasma (water is also filtered). However, plasma-protein binding generally does not limit renal tubular secretion or biotransformation, since these processes lower the free drug concentration, and this is rapidly followed by dissociation of the drug–protein complex. Drug transport and metabolism also are limited by plasma binding except when these are especially efficient and drug clearance, calculated on the basis of unbound drug, exceeds organ plasma flow. In this situation, binding of the drug to plasma protein may be viewed as a transport mechanism that fosters drug elimination by delivering drug to sites for elimination.

Tissue Binding. Many drugs accumulate in tissues at higher concentrations than those in the extracellular fluids and blood. For example, during long-term administration of the antimalarial agent quinacrine, the concentration of drug in the liver may be several thousandfold higher than

that in the blood. Such accumulation may be a result of active transport or, more commonly, binding. Tissue binding of drugs usually occurs with cellular constituents such as proteins, phospholipids, or nuclear proteins and generally is reversible. A large fraction of drug in the body may be bound in this fashion and serve as a reservoir that prolongs drug action in that same tissue or at a distant site reached through the circulation.

Fat as a Reservoir. Many lipid-soluble drugs are stored by physical solution in the neutral fat. In obese persons, the fat content of the body may be as high as 50%, and even in starvation it constitutes 10% of body weight; hence, fat can serve as an important reservoir for lipid-soluble drugs. For example, as much as 70% of the highly lipid-soluble barbiturate thiopental may be present in body fat 3 hours after administration. However, fat is a rather stable reservoir because it has a relatively low blood flow.

Bone. The tetracycline antibiotics (and other divalent-metal-ion chelating agents) and heavy metals may accumulate in bone by adsorption onto the bone-crystal surface and eventual incorporation into the crystal lattice. Bone can become a reservoir for the slow release of toxic agents such as lead or radium into the blood; their effects can thus persist long after exposure has ceased. Local destruction of the bone medulla also may lead to reduced blood flow and prolongation of the reservoir effect, since the toxic agent becomes sealed off from the circulation; this may further enhance the direct local damage to the bone. A vicious cycle results, whereby the greater the exposure to the toxic agent, the slower is its rate of elimination.

Redistribution. Termination of drug effect usually is by metabolism and excretion, but it also may result from redistribution of the drug from its site of action into other tissues or sites. Redistribution is a factor in terminating drug effect primarily when a highly lipid-soluble drug that acts on the brain or cardiovascular system is administered rapidly by intravenous injection or by inhalation. A good example of this is the use of the intravenous anesthetic thiopental, a highly lipid-soluble drug. Because blood flow to the brain is so high, the drug reaches its maximal concentration in brain within a minute after it is injected intravenously. After injection is concluded, the plasma concentration falls as thiopental diffuses into other tissues, such as muscle. The concentration of the drug in brain follows that of the plasma, because there is little binding of the drug to brain constituents. Thus, onset of anesthesia is rapid, but so is its termination. Both are directly related to the concentration of drug in the brain.

Central Nervous System and Cerebrospinal Fluid. The distribution of drugs into the CNS from the blood is unique, because functional barriers are present that restrict entry of drugs into this critical site. One reason for this is that the brain capillary endothelial cells have continuous tight junctions; therefore, drug penetration into the brain depends on transcellular rather than paracellular transport between cells. The unique characteristics of pericapillary glial cells also contribute to the blood–brain barrier. At the choroid plexus, a similar blood–cerebrospinal fluid (CSF) barrier is present except that it is epithelial cells that are joined by tight junctions rather than endothelial cells. As a result, the lipid solubility of the nonionized and unbound species of the drug is an important determinant of its uptake by the brain; the more lipophilic it is, the more likely it is to cross the blood–brain barrier. This situation often is used in drug design to alter brain distribution; for example, nonsedating antihistamines achieve far lower brain concentrations than do other agents in this class. Increasing evidence also indicates that drugs may penetrate into the CNS by specific uptake transporters normally involved in the transport of nutrients and endogenous compounds from blood into the brain and CSF. Recently, it has been discovered that another important factor in the functional blood–brain barrier also involves membrane transporters which are, in this case, efflux carriers present in the brain capillary endothelial cell. P-glycoprotein is the most important of these and functions by a combination of not allowing drug to even translocate across the endothelial cell and also by exporting any drug that enters the brain by other means. Such transport may account for the brain, and other tissues where P-glycoprotein is similarly expressed (*e.g.*, the testes), being pharmacological sanctuary sites where drug concentrations are below those necessary to achieve a desired effect even though blood levels are adequate. This situation apparently occurs with HIV protease inhibitors (Kim *et al.*, 1998) and also with loperamide—a potent, systemically active opioid that lacks any central effects characteristic of other opioids (*see* Chapter 23). Efflux transporters that actively secrete drug from the CSF into the blood also are present in the choroid plexus. Regardless of whether a drug is pumped out of the CNS by specific transporters or diffuses back into the blood, drugs also exit the CNS along with the bulk flow of CSF through the arachnoid villi. In general, the blood–brain barrier's function is well maintained; however, meningeal and encephalic inflammation increase the local permeability. There also is the potential that the blood–brain barrier may be advantageously modulated to enhance the treatment of infections or tumors in the brain. To date, however, such an approach has not been shown to be clinically useful.

Placental Transfer of Drugs. The potential transfer o drugs across the placenta is important, since drugs ma

cause congenital anomalies. Administered immediately before delivery, they also may have adverse effects on the neonate. Lipid solubility, extent of plasma binding, and degree of ionization of weak acids and bases are important general determinants, as previously discussed. The fetal plasma is slightly more acidic than that of the mother (pH 7.0 to 7.2 *versus* 7.4), so that ion-trapping of basic drugs occurs. As in the brain, P-glycoprotein is present in the placenta and functions as an export transporter to limit fetal exposure to potentially toxic agents. But the view that the placenta is an absolute barrier to drugs is inaccurate. A more appropriate approximation is that the fetus is to at least some extent exposed to essentially all drugs taken by the mother.

EXCRETION OF DRUGS

Drugs are eliminated from the body either unchanged by the process of excretion or converted to metabolites. Excretory organs, the lung excluded, eliminate polar compounds more efficiently than substances with high lipid solubility. Lipid-soluble drugs thus are not readily eliminated until they are metabolized to more polar compounds.

The kidney is the most important organ for excreting drugs and their metabolites. Substances excreted in the feces are mainly unabsorbed, orally ingested drugs or metabolites excreted either in the bile or secreted directly into the intestinal tract and, subsequently, not reabsorbed. Excretion of drugs in breast milk is important, not because of the amounts eliminated, but because the excreted drugs are potential sources of unwanted pharmacological effects in the nursing infant. Pulmonary excretion is important mainly for the elimination of anesthetic gases and vapors (*see* Chapters 13, 14, and 16); occasionally, small quantities of other drugs or metabolites are excreted by this route.

Renal Excretion. Excretion of drugs and metabolites in the urine involves three processes: glomerular filtration, active tubular secretion, and passive tubular reabsorption. Changes in overall renal function generally affect all three processes to a similar extent. Renal function is low compared to body size in neonates but rapidly matures within the first few months after birth. During adulthood there is a slow decline in renal function, about 1% per year, so that in the elderly a substantial degree of impairment is usually present.

The amount of drug entering the tubular lumen by filtration is dependent on the glomerular filtration rate and the extent of plasma binding of the drug; only unbound drug is filtered. In the proximal renal tubule, active, carrier-mediated tubular secretion also may add drug to the tubular fluid. Transporters such as P-glycoprotein and the multidrug resistance–associated protein-type 2 (MRP2) localized in the apical, brush-border membrane are largely responsible for the secretion of amphipathic anions and conjugated metabolites (such as glucuronides, sulfates, and glutathione adducts), respectively. Transport systems that are similar but more selective for organic cationic drugs (OCDs) are involved in the secretion of organic bases. Membrane transporters, mainly located in the distal renal tubule, also are responsible for any active reabsorption of drug from the tubular lumen back into the systemic circulation. However, most of such reabsorption occurs by nonionic diffusion.

In the proximal and distal tubules, the nonionized forms of weak acids and bases undergo net passive reabsorption. The concentration gradient for back-diffusion is created by the reabsorption of water with Na^+ and other inorganic ions. Since the tubular cells are less permeable to the ionized forms of weak electrolytes, passive reabsorption of these substances is pH-dependent. When the tubular urine is made more alkaline, weak acids are excreted more rapidly and to a greater extent, primarily because they are more ionized and passive reabsorption is decreased. When the tubular urine is made more acidic, the excretion of weak acids is reduced. Alkalinization and acidification of the urine have the opposite effects on the excretion of weak bases. In the treatment of drug poisoning, the excretion of some drugs can be hastened by appropriate alkalinization or acidification of the urine. Whether or not alteration of urine pH results in a significant change in drug elimination depends upon the extent and persistence of the pH change and the contribution of pH-dependent passive reabsorption to total drug elimination. The effect is greatest for weak acids and bases with pK_a values in the range of urinary pH (5 to 8). However, alkalinization of urine can produce a fourfold to sixfold increase in excretion of a relatively strong acid such as salicylate when urinary pH is changed from 6.4 to 8.0. The fraction of nonionized drug would decrease from 1% to 0.04%.

Biliary and Fecal Excretion. Transport systems analogous to those in the kidney also are present in the canalicular membrane of the hepatocyte, and these actively secrete drugs and metabolites into bile. P-glycoprotein transports a plethora of amphipathic, lipid-soluble drugs, whereas MRP2 is mainly involved in the secretion of conjugated metabolites of drugs (glutathione conjugates, glucuronides, and some sulfates). MRP2 also is involved in the excretion

of endogenous compounds, and the Dubin-Johnson syndrome is caused by a genetically determined absence of this transporter. Active biliary secretion of organic cations also involves transporters. Ultimately, drugs and metabolites present in bile are released into the intestinal tract during the digestive process. Because secretory transporters such as P-glycoprotein also are expressed on the apical membrane of enterocytes, direct secretion of drugs and metabolites may occur from the systemic circulation into the intestinal lumen. Subsequently, drugs and metabolites can be reabsorbed back into the body from the intestine which, in the case of conjugated metabolites like glucuronides, may require their enzymatic hydrolysis by the intestinal microflora. Such enterohepatic recycling, if extensive, may prolong significantly the presence of a drug and its effects within the body prior to elimination by other pathways.

Excretion by Other Routes. Excretion of drugs into sweat, saliva, and tears is quantitatively unimportant. Elimination by these routes is dependent mainly upon diffusion of the nonionized, lipid-soluble form of drugs through the epithelial cells of the glands and is pH-dependent. Drugs excreted in the saliva enter the mouth, where they are usually swallowed. The concentration of some drugs in saliva parallels that in plasma. Saliva therefore may be a useful biological fluid in which to determine drug concentrations when it is difficult or inconvenient to obtain blood. The same principles apply to excretion of drugs in breast milk. Since milk is more acidic than plasma, basic compounds may be slightly concentrated in this fluid, and the concentration of acidic compounds in the milk is lower than in plasma. Nonelectrolytes, such as ethanol and urea, readily enter breast milk and reach the same concentration as in plasma, independent of the pH of the milk. Although excretion into hair and skin also is quantitatively unimportant, sensitive methods of detection of drugs in these tissues have forensic significance.

METABOLISM OF DRUGS

The lipophilic characteristics of drugs that promote their passage through biological membranes and subsequent access to their site of action hinder their excretion from the body. Renal excretion of unchanged drug plays only a modest role in the overall elimination of most therapeutic agents, since lipophilic compounds filtered through the glomerulus are largely reabsorbed back into the systemic circulation during passage through the renal tubules. The metabolism of drugs and other xenobiotics into more hydrophilic metabolites is therefore essential for the elimination of these compounds from the body and termination of their biological activity. In general, biotransformation reactions generate more polar, inactive metabolites that are readily excreted from the body. However, in some cases, metabolites with potent biological activity or toxic properties are generated. Many of the metabolic biotransformation reactions leading to inactive metabolites of drugs also generate biologically active metabolites of endogenous compounds. The following discussion focuses on the biotransformation of drugs but is generally applicable to the metabolism of all xenobiotics as well as a number of endogenous compounds, including steroids, vitamins, and fatty acids.

Phase I and Phase II Metabolism. Drug biotransformation reactions are classified as either phase I functionalization reactions or phase II biosynthetic (conjugation) reactions. Phase I reactions introduce or expose a functional group on the parent compound. Phase I reactions generally result in the loss of pharmacological activity, although there are examples of retention or enhancement of activity. In rare instances, metabolism is associated with an altered pharmacological activity. Prodrugs are pharmacologically inactive compounds, designed to maximize the amount of the active species that reaches its site of action. Inactive prodrugs are converted rapidly to biologically active metabolites, often by the hydrolysis of an ester or amide linkage. If not rapidly excreted into the urine, the products of phase I biotransformation reactions can then react with endogenous compounds to form a highly water-soluble conjugate.

Phase II conjugation reactions lead to the formation of a covalent linkage between a functional group on the parent compound or phase I metabolite with endogenously derived glucuronic acid, sulfate, glutathione, amino acids, or acetate. These highly polar conjugates are generally inactive and are excreted rapidly in the urine and feces. An example of an active conjugate is the 6-glucuronide metabolite of morphine, which is a more potent analgesic than its parent compound.

Site of Biotransformation. The metabolic conversion of drugs generally is enzymatic in nature. The enzyme systems involved in the biotransformation of drugs are localized in the liver, although every tissue examined has some metabolic activity. Other organs with significant metabolic capacity include the gastrointestinal tract, kidneys, and lungs. Following nonparenteral administration of a drug, a significant portion of the dose may be metabolically inactivated in either the intestinal epithelium or the liver before it reaches the systemic circulation. This first-pass metabolism significantly limits the oral availability of highly metabolized drugs. Within a given cell, most drug-metabolizing activity is found in the endoplasmic reticulum and the cytosol, although drug biotransformations also can occur in the mitochondria, nuclear envelope, and

plasma membrane. Upon homogenization and differential centrifugation of tissues, the endoplasmic reticulum breaks up, and fragments of the membrane form microvesicles, referred to as microsomes. The drug-metabolizing enzymes in the endoplasmic reticulum therefore often are classified as microsomal enzymes. The enzyme systems involved in phase I reactions are located primarily in the endoplasmic reticulum, while the phase II conjugation enzyme systems are mainly cytosolic. Often drugs biotransformed through a phase I reaction in the endoplasmic reticulum are conjugated at this same site or in the cytosolic fraction of the same cell.

Cytochrome P450 Monooxygenase System. The cytochrome P450 enzymes are a superfamily of heme-thiolate proteins widely distributed across all living kingdoms. The enzymes are involved in the metabolism of a plethora of chemically diverse, endogenous and exogenous compounds, including drugs, environmental chemicals, and other xenobiotics. Usually they function as a terminal oxidase in a multicomponent electron-transfer chain that introduces a single atom of molecular oxygen into the substrate with the other atom being incorporated into water. In microsomes, the electrons are supplied from NADPH *via* cytochrome P450 reductase, which is closely associated with cytochrome P450 in the lipid membrane of the smooth endoplasmic reticulum. Cytochrome P450 catalyzes many reactions, including aromatic and side-chain hydroxylation; *N*-, *O*- and *S*-dealkylation; *N*-oxidation; *N*-hydroxylation; sulfoxidation; deamination; dehalogenation; and desulfuration. Details and examples of cytochrome P450–mediated metabolism are shown in Table 1–2. A number of reductive reactions also are catalyzed by these enzymes, generally under conditions of low oxygen tension.

Of the approximately 1000 currently known cytochrome P450s, about 50 are functionally active in human beings. These are categorized into 17 families and many subfamilies according to the amino acid–sequence similarities of the predicted proteins; the abbreviated term *CYP* is used for identification. Sequences that are greater than 40% identical belong to the same family, identified by an Arabic number; within a family, sequences greater than 55% identical are in the same subfamily, identified by a letter; and different individual isoforms within the subfamily are identified by an Arabic number. About 8 to 10 isoforms in the CYP1, CYP2, and CYP3 families primarily are involved in the majority of all drug metabolism reactions in human beings; members of the other families are important in the biosynthesis and degradation of steroids, fatty acids, vitamins, and other endogenous compounds. Each individual CYP isoform appears to have a characteristic substrate specificity based on structural features of the substrate; considerable overlap, however, often is present. As a result, two or more CYP isoforms and other drug-metabolizing enzymes often are involved in a drug's overall metabolism, leading to the formation of many primary and secondary metabolites. The various isoforms also have characteristic inhibition and induction profiles, as described later. Additionally, CYP-catalyzed metabolism is often regio- and stereoselective; the latter characteristic may be important if the administered drug is a racemate and the enantiomers have different pharmacological activities.

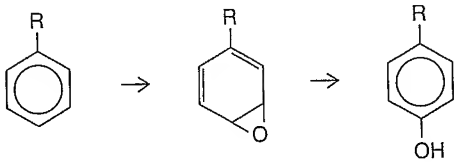
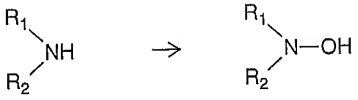
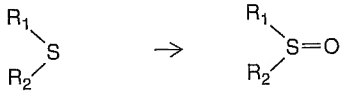
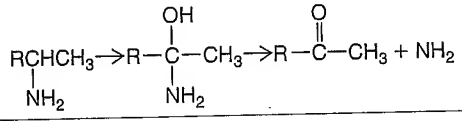
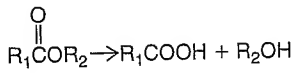
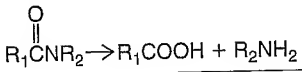
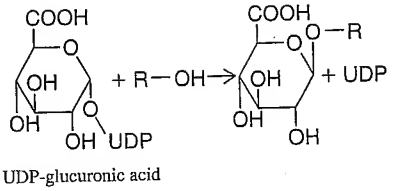
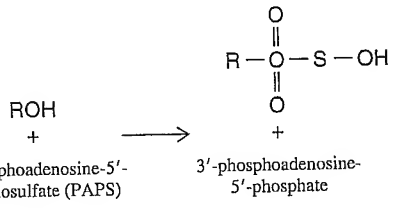
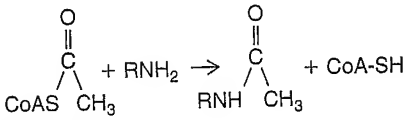
The relative contributions of the various CYP isoforms in the metabolism of drugs is illustrated in Figure 1–3. CYP3A4 and CYP3A5, which are very similar isoforms, together are involved in the metabolism of about 50% of drugs; moreover, CYP3A is expressed in both the intestinal epithelium and the kidney. It is now recognized that metabolism by CYP3A during absorption through the intestinal enterocyte is a significant factor, along with hepatic first-pass metabolism, in the poor oral bioavailability of many drugs. Isoforms in the CYP2C family and CYP2D6 subfamily also are involved to a large extent in the metabolism of drugs. Although isoforms such as CYP1A1/2, CYP2A6, CYP2B1, and CYP2E1 are not involved to any major extent in the metabolism of therapeutic drugs, they do, however, catalyze the activation of many procarcinogenic environmental chemicals to the ultimate carcinogenic form. Accordingly, they are considered to be important in susceptibility to various cancers, such as tobacco smoking–associated lung cancer.

Other oxidative enzymes such as dehydrogenases and flavin-containing monooxygenases also are capable of catalyzing the metabolism of specific drugs, but, in general, such enzymes are of minor overall importance.

Hydrolytic Enzymes. The reactions of the major hydrolytic enzymes are illustrated in Table 1–2. A number of nonspecific esterases and amidases have been identified in the endoplasmic reticulum of human liver, intestine, and other tissues. The alcohol and amine groups exposed following hydrolysis of esters and amides are suitable substrates for conjugation reactions. Microsomal epoxide hydrolase is found in the endoplasmic reticulum of essentially all tissues and is in close proximity to the cytochrome P450 enzymes. Epoxide hydrolase generally is considered a detoxification enzyme, hydrolyzing highly reactive arene oxides generated from cytochrome P450 oxidation reactions to inactive, water-soluble transdihydrodiol metabolites. Protease and peptidase enzymes are widely distributed in many tissues and are involved in the biotransformation of polypeptide drugs. Delivery of such drugs across biological membranes requires the inhibition of these enzymes or the development of stable analogs.

Conjugation Reactions. Both an activated form of an endogenous compound and an appropriate transferase enzyme are necessary for the formation of a conjugated metabolite. In the case of glucuronidation—the most important conjugation reaction (Figure 1–3)—uridine diphosphate glucuronosyltransferases (UGTs) catalyze the transfer of glucuronic acid to aromatic and aliphatic alcohols, carboxylic acids, amines, and free sulfhydryl groups of both exogenous and endogenous compounds to form *O*-, *N*-, and *S*-glucuronides, respectively. Glucuronidation also is important in the elimination of endogenous steroids, bilirubin, bile acids, and fat-soluble vitamins. The increased water solubility of a glucuronide conjugate promotes its elimination in the urine or bile. Unlike most phase II reactions, which are localized in the cytosol, UGTs are microsomal enzymes. This location facilitates direct access of phase I metabolites formed at the same site. In addition to the liver, UGTs also are found in the intestinal epithelium, kidney, and skin. About 15 human UGTs have been identified, and, based on amino acid similarity (>50% identity), two main families have been categorized. Members of the

Table 1–2
Major Reactions Involved in Drug Metabolism

	Reaction	*Examples*
I. OXIDATIVE REACTIONS		
N-Dealkylation	$RNHCH_3 \rightarrow RNH_2 + CH_2O$	Imipramine, diazepam, codeine, erythromycin, morphine, tamoxifen, theophylline, caffeine
O-Dealkylation	$ROCH_3 \rightarrow ROH + CH_2O$	Codeine, indomethacin, dextromethorphan
Aliphatic hydroxylation	$RCH_2CH_3 \rightarrow RCH(OH)CH_3$	Tolbutamide, ibuprofen, pentobarbital, meprobamate, cyclosporine, midazolam
Aromatic hydroxylation		Phenytoin, phenobarbital, propanolol, phenylbutazone, ethinylestradiol, amphetamine, warfarin
N-Oxidation	$RNH_2 \rightarrow RNHOH$	Chlorpheniramine, dapsone, meperidine
		Quinidine, acetaminophen
S-Oxidation		Cimetidine, chlorpromazine, thioridazine, omeprazole
Deamination		Diazepam, amphetamine
II. HYDROLYSIS REACTIONS		
		Procaine, aspirin, clofibrate, meperidine, enalapril, cocaine
		Lidocaine, procainamide, indomethacin
III. CONJUGATION REACTIONS		
Glucuronidation	 UDP-glucuronic acid	Acetaminophen, morphine, oxazepam, lorazepam
Sulfation	 3′-phosphoadenosine-5′-phosphosulfate (PAPS) 3′-phosphoadenosine-5′-phosphate	Acetaminophen, steroids, methyldopa
Acetylation	 acetyl-coenzyme A	Sulfonamides, isoniazid, dapsone, clonazepa

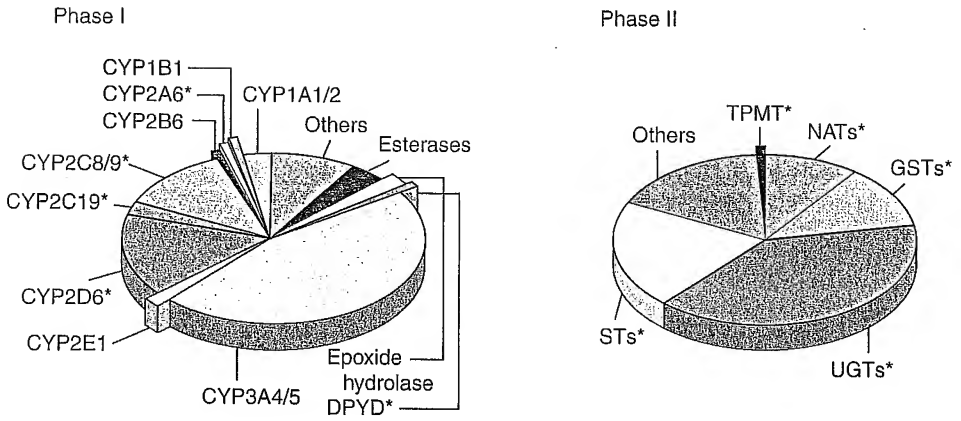


Figure 1–3. The proportion of drugs metabolized by the major phase I and phase II enzymes.

The relative size of each pie section indicates the estimated percentage of phase I (*left panel*) or phase II (*right panel*) metabolism that each enzyme contributes to the metabolism of drugs based on literature reports. Enzymes that have functional allelic variants are indicated by an asterisk. In many cases, more than one enzyme is involved in a particular drug's metabolism: CYP, cytochrome P450; DPYD, dihydropyrimidine dehydrogenase; GST, glutathione *S*-transferases; NAT, *N*-acetyltransferases; ST, sulfotransferases; TPMT, thiopurine methyltransferase; UGT, UDP-glucuronosyltransferases.

human UGT1A family are all encoded by a complex gene, and individual isoforms are produced by alternative splicing of 12 promoters/exon 1 with common exons 2 to 5 to produce multiple different proteins. By contrast, UGT2 contains only three subfamilies: 2A, 2B, and 2C. While it appears that individual UGTs have characteristic substrate specificities, there is considerable overlap, so that multiple isoforms may be responsible for formation of a particular glucuronide metabolite. Cytosolic sulfation also is an important conjugation reaction that involves the catalytic transfer by sulfotransferases (STs) of inorganic sulfur from activated 3′-phosphoadenosine-5′-phosphosulfate to the hydroxyl group of phenols and aliphatic alcohols. Therefore, drugs and primary metabolites with a hydroxyl group often form both glucuronide and sulfate metabolites. Two *N*-acetyltransferases (NAT1 and NAT2) are involved in the acetylation of amines, hydrazines, and sulfonamides. In contrast to most drug conjugates, acetylated metabolites often are less water-soluble than the parent drug, and this may result in crystalluria unless a high urine flow rate is maintained.

Factors Affecting Drug Metabolism. A hallmark of drug metabolism is a large interindividual variability that often results in marked differences in the extent of metabolism and, as a result, the drug's rate of elimination and other characteristics of its plasma concentration–time profile. Such variability is a major reason why patients differ in their responses to a standard dose of a drug and it must be considered in optimizing a dosage regimen for a particular individual. A combination of genetic, environmental, and disease-state factors affect drug metabolism, with the relative contribution of each depending on the specific drug.

Genetic Variation. Advances in molecular biology have shown that genetic diversity is the rule rather than the exception with all proteins, including enzymes that catalyze drug-metabolism reactions. For an increasing number of such enzymes, allelic variants with different catalytic activities from that of the wild-type form have been identified. The differences involve a variety of molecular mechanisms leading to a complete lack of activity, a reduction in catalytic ability, or, in the case of gene duplication, enhanced activity. Furthermore, these traits are generally inherited in an autosomal, Mendelian recessive fashion and, if sufficiently prevalent, result in subpopulations with different drug-metabolizing abilities, *i.e., genetic polymorphism.* In addition, the frequency of specific allelic variants often varies according to the racial ancestry of the individual. It is possible to phenotype or genotype a person with respect to a particular genetic variant, and it is likely that such characterization will become increasingly useful in individualizing drug therapy, especially for drugs with a narrow therapeutic index. Accumulating evidence also suggests that individual susceptibility to diseases associated with environmental chemicals, such as cancer, may reflect genetic variability in drug-metabolizing enzymes.

A number of genetic polymorphisms are present in several cytochrome P450s that lead to altered drug metabolizing ability. The best characterized of these is that associated with CYP2D6. About 70 *single nucleotide polymorphisms* (SNPs) and other genetic variants of functional importance have been identified in the CYP2D6 gene, many of which result in an inactive enzyme while others reduce catalytic activity; gene duplication also occurs. As a result, four phenotypic subpopulations of metabolizers exist: poor (PM), intermediate (IM), extensive (EM),

and ultrarapid (UM). Some of the variants are relatively rare, whereas others are more common, and importantly, their frequency varies according to racial background. For example, 5% to 10% of Caucasians of European ancestry are PMs, whereas the frequency of this homozygous phenotype in individuals of Southeast Asian origin is only about 1% to 2%. More than 65 commonly used drugs are metabolized by CYP2D6, including tricyclic antidepressants, neuroleptic agents, selective serotonin reuptake inhibitors, some antiarrhythmic agents, β-adrenergic receptor antagonists, and certain opiates. The clinical importance of the CYP2D6 polymorphism is mainly in the greater likelihood of an adverse reaction in PMs when the affected metabolic pathway is a major contributor to the drug's overall elimination. Also, in UMs, usual drug doses may be inefficacious, or in the case where an active metabolite is formed, for example, the CYP2D6-catalyzed formation of morphine from codeine, an exaggerated response occurs. Inhibitors of CYP2D6, such as quinidine and selective serotonin reuptake inhibitors, may convert a genotypic EM into a phenotypic PM, a phenomenon termed *phenocopying* that is an important aspect of drug interactions with this particular CYP isoform.

CYP2C9 catalyzes the metabolism of some 16 commonly used drugs, including that of warfarin and phenytoin, both of which have a narrow therapeutic index. The two most common allelic CYP2C9 variants have markedly reduced catalytic activity (5% to 12%) compared to the wild-type enzyme. As a consequence, patients who are heterozygous or homozygous for the mutant alleles require a lower anticoagulating dose of warfarin, especially the latter group, relative to homozygous, wild-type individuals. Also, initiating warfarin therapy is more difficult, and there is an increased risk of bleeding complications. Similarly, high plasma concentrations of phenytoin and associated adverse effects occur in patients with variant CYP2C9 alleles. Genetic polymorphism also occurs with CYP2C19, where 8 allelic variants have been identified that result in a catalytically inactive protein. About 3% of Caucasians are phenotypically PMs, whereas the frequency is far higher in Southeast Asians, 13% to 23%. Proton-pump inhibitors such as omeprazole and lansoprazole are among the 18 or so drugs importantly metabolized by CYP2C19 to an extent determined by the gene dose. The efficacy of the recommended 20-mg dose of omeprazole in combination with amoxicillin in eradicating *Helicobacter pylori* is markedly reduced in patients of the homozygous wild-type genotype compared with the 100% cure rate in homozygous PMs, reflecting differences in the drug's effect on gastric acid secretion. Although CYP3A activity shows marked interindividual variability (>10-fold), no significant functional polymorphisms have been found in the gene's coding region; it is, therefore, likely that unknown regulatory factors primarily determine such variability. Genetic variability also is present with dihydropyrimidine dehydrogenase (DPYD), which is a key enzyme in the metabolism of 5-fluorouracil. Accordingly, there is a marked risk of developing severe drug-induced toxicity in the 1% to 3% of cancer patients treated with this antimetabolite who have substantially reduced DPYD activity compared to the general population.

A polymorphism in a conjugating drug-metabolizing enzyme, namely that in NAT2, was one of the first to be found to have a genetic basis some 50 years ago. This isoform is involved in the metabolism of about 16 common drugs including isoniazid, procainamide, dapsone, hydralazine, and caffeine. About 15 allelic variants have been identified, some of which are without functional effect, but others are associated with either reduced or absent catalytic activity. Considerable heterogeneity is present in the worldwide population frequency of these alleles, so that the slow-acetylator phenotype frequency is about 50% in American whites and blacks, 60% to 70% in North Europeans, but only 5% to 10% in Southeast Asians. It has been speculated that acetylator phenotype may be associated with environmental agent–induced disease such as bladder and colorectal cancer; however, definitive evidence is not yet available. Similarly, genetic variability in the catalytic activity of glutathione *S*-transferases may be linked to individual susceptibility to such diseases. Thiopurine methyltransferase (TPMT) is critically important in the metabolism of 6-mercaptopurine, the active metabolite of azathioprine. As a result, homozygotes for alleles encoding inactive TPMT (0.3% to 1% of the population) predictably exhibit severe pancytopenia if given standard doses of azathioprine; such patients typically can be treated with 10% to 15% of the usual dose.

Environmental Determinants. The activity of most drug-metabolizing enzymes may be modulated by exposure to certain exogenous compounds. In some instances, this may be a drug, which, if concomitantly administered with a second agent, results in a drug:drug interaction. Additionally, dietary micronutrients and other environmental factors can up- or down-regulate the enzymes, termed *induction* and *inhibition,* respectively. Such modulation is thought to be a major contributor to interindividual variability in the metabolism of many drugs.

Inhibition of Drug Metabolism. A consequence of inhibiting drug-metabolizing enzymes is an increase in the plasma concentration of parent drug and a reduction in that of metabolite, exaggerated and prolonged pharmacological effects, and an increased likelihood of drug-induced toxicity. These changes occur rapidly and with essentially no warning and are most critical for drugs that are extensively metabolized and have a narrow therapeutic index. Knowledge of the cytochrome-P450 isoforms that catalyze the main pathway of metabolism of a drug provides a basis for predicting and understanding inhibition, especially with regard to drug-drug interactions. This is because many inhibitors are more selective for some isoforms than others. Often, inhibition occurs because of competition between two or more substrates for the same active site of the enzyme, the extent of which depends on the relative concentrations of the substrates and their affinities for the enzyme. In certain instances, however, the enzyme may be irreversibly inactivated; for example, the substrate or a metabolite forms a tight complex with the heme iron of cytochrome P450 (cimetidine, ketoconazole) or the heme group may be destroyed (norethindrone, ethinylestradiol). A common mechanism of inhibition for some phase II enzymes is the depletion of necessary cofactors.

Inhibition of the CYP3A-catalyzed mechanism is both common and important. Because of the high expression level of CYP3A in the intestinal epithelium and the fact that oral ingestion is the most common route of entry of drugs and environmental agents into the body, inhibition of the isoform's activity at this site is often particularly consequential, even if

that in the liver is unaffected. This is because of the potential, large increase in bioavailability associated with the reduction in first-pass metabolism for drugs that usually exhibit this effect to a substantial extent. The antifungal agents ketoconazole and itraconazole, HIV protease inhibitors (especially ritonavir), macrolide antibiotics such as erythromycin and clarithromycin but not azithromycin, are all potent CYP3A inhibitors. Certain calcium channel blockers such as diltiazem, nicardipine, and verapamil also inhibit CYP3A, as does a constituent of grapefruit juice. Many inhibitors of CYP3A also reduce P-glycoprotein function, so that drug-drug interactions may involve a dual mechanism. Also, the disposition of drugs that are not significantly metabolized but are eliminated by P-glycoprotein–mediated transport also may be affected by a CYP3A inhibitor. For example, the impaired excretion of digoxin by quinidine and a large number of other unrelated drugs is caused by inhibition of P-glycoprotein. With CYP2D6, quinidine and selective serotonin reuptake inhibitors are potent inhibitors that may produce phenocopying. On the other hand, other drugs are more general inhibitors of cytochrome P450–catalyzed metabolism. For example amiodarone, cimetidine (but not ranitidine), paroxitene, and fluoxetine reduce the metabolic activity of several CYP isoforms. Phase I metabolic enzymes other than cytochrome P450 also may be inhibited by drug administration, as exemplified by the potent effect of valproic acid on microsomal epoxide hydrolase, and the inhibition of xanthine oxidase by allopurinol, which can result in life-threatening toxicity in patients concurrently receiving 6-mercaptopurine.

Induction of Drug Metabolism. Up-regulation of drug-metabolizing activity usually occurs by enhanced gene transcription following prolonged exposure to an inducing agent, although with CYP2E1 stabilization of the protein against degradation is the major mechanism. As a result, the consequences of induction take considerable time to be fully exhibited, *c.f.*, inhibition of metabolism. Moreover, the consequences of induction are an increased rate of metabolism, enhanced oral first-pass metabolism and reduced bioavailability, and a corresponding decrease in the drug's plasma concentration, all factors that reduce drug exposure. By contrast, for drugs that are metabolized to an active or reactive metabolite, induction may be associated with increased drug effects or toxicity, respectively. In some cases, a drug can induce both the metabolism of other compounds and its own metabolism; such *autoinduction* occurs with the anticonvulsant carbamazepine. In many cases involving induction, the dosage of an affected drug must be increased to maintain the therapeutic effect. This is particularly the case when induction is extensive following administration of a highly effective inducer; in fact, women are advised to use an alternative to oral contraceptives for birth control during rifampin therapy because efficacy cannot be assured. The therapeutic risk associated with metabolic induction is most critical when administration of the inducing agent is stopped while maintaining the same dose of a drug that has been previously given. In this case, as the inducing effect wears off, plasma concentrations of the second drug will rise unless the dose is reduced, with an increase in the potential for adverse effects.

Inducers generally are selective for certain CYP subfamilies and isoforms, but at the same time, multiple other enzymes may be simultaneously up-regulated through a common molecular mechanism. For example, polycyclic aromatic hydrocarbons derived from environmental pollutants, cigarette smoke, and charbroiled meats produce marked induction of the CYP1A subfamily of enzymes both in the liver and extrahepatically. This involves activation of the cytosolic arylhydrocarbon receptor (AhR), which interacts with another regulatory protein, the AhR nuclear translocator (Arnt); the complex functions as a transcription factor to up-regulate CYP1A expression. In addition, the expression of phase II enzymes such as UGTs, GSTs, and NAD(P)H:quinone oxidoreductase are simultaneously increased. A similar type of receptor mechanism involving the pregnane X receptor (PXR) is involved in the induction of CYP3A by a wide variety of diverse chemicals, including drugs such as rifampin and rifabutin, barbiturates and other anticonvulsants, some glucocorticoids, and even alternative medicines such as St. John's wort. These latter drugs also can affect other CYP isoforms; for example, rifampin and carbamazepine induce CYP1A2, CYP2C9, and CYP2C19. Chronic alcohol use also results in enzyme induction, especially with CYP2E1; the risk of hepatotoxic adverse effects of acetaminophen is higher in alcoholics because of increased CYP2E1-mediated formation of a reactive metabolite, *N*-acetyl-*p*-benzoquinoneimine.

Disease Factors. Since the liver is the major location of drug-metabolizing enzymes, dysfunction in this organ in patients with hepatitis, alcoholic liver disease, biliary cirrhosis, fatty liver, and hepatocarcinomas potentially can lead to impaired drug metabolism. In general, the severity of the liver damage determines the extent of reduced metabolism; unfortunately, common clinical tests of liver function are of little value in assessing this. Moreover, even in severe cirrhosis, the extent of impairment is only to about 30% to 50% of the activity in non-liver-diseased patients. However, with drugs that undergo substantial hepatic first-pass metabolism, oral bioavailability may be increased two- to fourfold in liver disease which, coupled with the prolonged presence of drugs in the body, increases the risk of exaggerated pharmacological responses and adverse effects. It appears that cytochrome-P450 isoforms are affected to a greater extent by liver disease than are those that catalyze phase II reactions such as glucuronosyltransferases.

Severe cardiac failure and shock can result in both decreased perfusion of the liver and impaired metabolism. The best example of this is the almost twofold reduction in lidocaine metabolism in cardiac failure, which also is accompanied by a change in distribution to a similar extent. As a result, the loading and maintenance doses of lidocaine used to treat cardiac arrhythmias in such patients are substantially different from those used in patients without this condition.

Age and Sex. Functional cytochrome P450 isoforms and to a lesser degree phase II drug-metabolizing enzymes develop early in fetal development, but the levels, even at birth, are lower than those found postnatally. Both phase I and phase II enzymes begin to mature gradually following the first 2 to 4 weeks postpartum, although the pattern of development is variable for the different enzymes. Thus, newborns and infants are able to metabolize drugs relatively efficiently but generally at a slower rate than are adults. An exception to this is the impairment of bilirubin glucuronidation at birth, which contributes to the hyperbilirubinemia of newborns. Full maturity appears to occur in the second decade of life with a subsequent slow decline in function associated with aging. Unfortunately, few generalizations are possible regarding the extent or clinical importance of such age-related changes in an individual patient. This is particularly true for elderly patients who,

because of multiple diseases, may be taking a large number of drugs, many of which may produce drug-drug interactions. In addition, increased sensitivity of target organs and impairment of physiological control mechanisms further complicate the use of drugs in the elderly population. Phase I drug-metabolizing enzymes appear to be affected to a greater extent than are those that catalyze phase II reactions. However, the changes are often modest relative to other causes of interindividual variability in metabolism. On the other hand, for drugs exhibiting a high first-pass effect, even a small reduction in metabolizing ability may significantly increase oral bioavailability. Drug use in the elderly, therefore, generally requires moderate reductions in drug dose and awareness of the possibility of exaggerated pharmacodynamic responsiveness.

A number of examples indicate that drug treatment and/or responsiveness of men and women may be different for certain drugs. Some sex-related differences in drug-metabolizing activity, especially that catalyzed by CYP3A, also have been noted. However, such differences are minor and unimportant relative to other factors involved in interindividual variability in metabolism. One exception to this generalization is pregnancy, where induction of certain drug-metabolizing enzymes occurs in the second and third trimesters. As a result, drug dosage may have to be increased during this period and returned to its previous level postpartum. This situation is particularly important in the treatment of patients with seizures using phenytoin during their pregnancy. Many oral contraceptive agents also are potent irreversible inhibitors of CYP isoforms through a suicide-inactivation mechanism.

CLINICAL PHARMACOKINETICS

A fundamental hypothesis of clinical pharmacokinetics is that a relationship exists between the pharmacological effects of a drug and an accessible concentration of the drug (*e.g.*, in blood or plasma). This hypothesis has been documented for many drugs, although for some drugs no clear or simple relationship has been found between pharmacological effect and concentration in plasma. In most cases, as depicted in Figure 1–1, the concentration of drug in the systemic circulation will be related to the concentration of drug at its sites of action. The pharmacological effect that results may be the clinical effect desired, a toxic effect, or, in some cases, an effect unrelated to therapeutic efficacy or toxicity. Clinical pharmacokinetics attempts to provide both a quantitative relationship between dose and effect and a framework with which to interpret measurements of concentrations of drugs in biological fluids. The importance of pharmacokinetics in patient care is based on the improvement in therapeutic efficacy that can be attained by application of its principles when dosage regimens are chosen and modified.

The various physiological and pathophysiological variables that dictate adjustment of dosage in individual patients often do so as a result of modification of pharmacokinetic parameters. The four most important parameters are *clearance,* a measure of the body's efficiency in eliminating drug; *volume of distribution,* a measure of the apparent space in the body available to contain the drug; *elimination half-life,* a measure of the rate of removal of drug from the body; and *bioavailability,* the fraction of drug absorbed as such into the systemic circulation. Of lesser importance are the *rates* of availability and distribution of the agent.

Clearance

Clearance is the most important concept that needs to be considered when a rational regimen for long-term drug administration is to be designed. The clinician usually wants to maintain steady-state concentrations of a drug within a *therapeutic window* associated with therapeutic efficacy and a minimum of toxicity. Assuming complete bioavailability, the steady state will be achieved when the rate of drug elimination equals the rate of drug administration:

$$\text{Dosing rate} = CL \cdot C_{ss} \tag{1–1}$$

where CL is clearance from the systemic circulation and C_{ss} is the steady-state concentration of drug. Thus, if the desired steady-state concentration of drug in plasma or blood is known, the rate of clearance of drug by the patient will dictate the rate at which the drug should be administered.

The concept of clearance is extremely useful in clinical pharmacokinetics, because its value for a particular drug usually is constant over the range of concentrations encountered clinically. This is true because systems for elimination of drugs such as metabolizing enzymes and transporters usually are not saturated, and thus the *absolute* rate of elimination of the drug is essentially a linear function of its concentration in plasma. A synonymous statement is that the elimination of most drugs follows first-order kinetics—a constant *fraction* of drug in the body is eliminated per unit of time. If mechanisms for elimination of a given drug become saturated, the kinetics approach zero-order—a constant *amount* of drug is eliminated per unit of time. Under such a circumstance, clearance will vary with the concentration of drug, often according to the following equation:

$$CL = v_m/(K_m + C) \tag{1–2}$$

where K_m represents the concentration at which half of the maximal rate of elimination is reached (in units of mass/volume) and v_m is equal to the maximal rate of elimination

(in units of mass/time). This equation is analogous to the Michaelis–Menten equation for enzyme kinetics. Design of dosage regimens for such drugs is more complex than when elimination is first-order and clearance is independent of the drug's concentration (*see* below).

Principles of drug clearance are similar to those of renal physiology, where, for example, creatinine clearance is defined as the rate of elimination of creatinine in the urine relative to its concentration in plasma. At the simplest level, clearance of a drug is its rate of elimination by all routes normalized to the concentration of drug, C, in some biological fluid:

$$CL = \text{rate of elimination}/C \tag{1–3}$$

Thus, when clearance is constant, the rate of drug elimination is directly proportional to drug concentration. It is important to note that clearance does not indicate how much drug is being removed but, rather, the volume of biological fluid such as blood or plasma from which drug would have to be completely removed to account for the elimination. Clearance is expressed as a volume per unit of time. Clearance usually is further defined as blood clearance (CL_b), plasma clearance (CL_p), or clearance based on the concentration of unbound drug (CL_u), depending on the concentration measured (C_b, C_p, or C_u).

Clearance by means of various organs of elimination is additive. Elimination of drug may occur as a result of processes that occur in the kidney, liver, and other organs. Division of the rate of elimination by each organ by a concentration of drug (*e.g.*, plasma concentration) will yield the respective clearance by that organ. Added together, these separate clearances will equal systemic clearance:

$$CL_{renal} + CL_{hepatic} + CL_{other} = CL \tag{1–4}$$

Other routes of elimination could include that in saliva or sweat, secretion into the intestinal tract, and metabolism at other sites.

Systemic clearance may be determined at steady state by using Equation (1–1). For a single dose of a drug with complete bioavailability and first-order kinetics of elimination, systemic clearance may be determined from mass balance and the integration of Equation (1–3) over time.

$$CL = \text{Dose}/AUC \tag{1–5}$$

where AUC is the total area under the curve that describes the concentration of drug in the systemic circulation as a function of time (from zero to infinity).

Examples. In Appendix II, the plasma clearance for cephalexin is reported as 4.3 $ml \cdot min^{-1} \cdot kg^{-1}$, with 90% of the drug excreted unchanged in the urine. For a 70-kg man, the clearance from plasma would be 300 ml/minute, with renal clearance accounting for 90% of this elimination. In other words, the kidney is able to excrete cephalexin at a rate such that it is completely removed (cleared) from approximately 270 ml of plasma per minute. Because clearance usually is assumed to remain constant in a stable patient, the rate of elimination of cephalexin will depend on the concentration of drug in the plasma [Equation (1–3)]. Propranolol is cleared from the blood at a rate of 16 $ml \cdot min^{-1} \cdot kg^{-1}$ (or 1120 ml/minute in a 70-kg man), almost exclusively by the liver. Thus, the liver is able to remove the amount of drug contained in 1120 ml of blood per minute. Even though the liver is the dominant organ for elimination, the plasma clearance of some drugs exceeds the rate of plasma (and blood) flow to this organ. Often this is because the drug partitions readily into red blood cells, and the rate of drug delivered to the eliminating organ is considerably higher than suspected from measurement of its concentration in plasma. The relationship between plasma and blood clearance at steady state is given by:

$$\frac{CL_p}{CL_b} = \frac{C_b}{C_p} = 1 + H\left[\frac{C_{rbc}}{C_p} - 1\right] \tag{1–6}$$

Clearance from the blood, therefore, may be estimated by dividing the plasma clearance by the drug's blood to plasma concentration ratio, obtained from knowledge of the hematocrit ($H = 0.45$) and the red cell to plasma concentration ratio. In most instances the blood clearance will be less than liver blood flow (1.5 liters/minute) or, if renal excretion also is involved, the sum of the two eliminating organs' blood flows. For example, the plasma clearance of tacrolimus, about 2 liters/minute, is more than twofold higher than the hepatic plasma flow rate and even exceeds the organ's blood flow, despite the fact that the liver is the predominant site of this drug's extensive metabolism. However, after taking into account the extensive distribution of tacrolimus into red cells, its clearance from the blood is only about 63 ml/minute, and it is actually a low- rather than high-clearance drug, as might be interpreted from the plasma clearance value. Sometimes, however, clearance from the blood by metabolism exceeds liver blood flow, and this indicates extrahepatic metabolism. In the case of esmolol (11.9 liters/minute), the blood clearance value is greater than cardiac output, because the drug is efficiently metabolized by esterases present in red blood cells.

A further definition of clearance is useful for understanding the effects of pathological and physiological variables on drug elimination, particularly with respect to an individual organ. The rate of presentation of drug to the organ is the product of blood flow (Q) and the arterial drug concentration (C_A), and the rate of exit of drug from the organ is the product of blood flow and the venous drug concentration (C_V). The difference between these rates at steady state is the rate of drug elimination:

$$\begin{aligned}\text{Rate of elimination} &= Q \cdot C_A - Q \cdot C_V \\ &= Q(C_A - C_V)\end{aligned} \tag{1–7}$$

Division of Equation (1–7) by the concentration of drug entering

the organ of elimination, C_A, yields an expression for clearance of the drug by the organ in question:

$$CL_{organ} = Q\left[\frac{C_A - C_V}{C_A}\right] = Q \cdot E \qquad (1\text{–}8)$$

The expression $(C_A - C_V)/C_A$ in Equation (1–8) can be referred to as the extraction ratio for the drug (E).

Hepatic Clearance. The concepts developed in Equation (1–8) have important implications for drugs that are eliminated by the liver. Consider a drug that is efficiently removed from the blood by hepatic processes—metabolism and/or excretion of drug into the bile. In this instance, the concentration of drug in the blood leaving the liver will be low, the extraction ratio will approach unity, and the clearance of the drug from blood will become limited by hepatic blood flow. Drugs that are cleared efficiently by the liver (*e.g.*, drugs in Appendix II with systemic clearances greater than 6 $ml \cdot min^{-1} \cdot kg^{-1}$, such as diltiazem, imipramine, lidocaine, morphine, and propranolol) are restricted in their rate of elimination, not by intrahepatic processes, but by the rate at which they can be transported in the blood to the liver.

Additional complexities also have been considered. For example, the equations presented above do not account for drug binding to components of blood and tissues, nor do they permit an estimation of the intrinsic ability of the liver to eliminate a drug in the absence of limitations imposed by blood flow, termed *intrinsic clearance*. In biochemical terms and under first-order conditions, intrinsic clearance is a measure of the ratio of the Michaelis–Menten kinetic parameters for the eliminating process, *i.e.*, v_m/K_m. Extensions of the relationships of Equation (1–8) to include expressions for protein binding and intrinsic clearance have been proposed for a number of models of hepatic elimination (*see* Morgan and Smallwood, 1990). All of these models indicate that, when the capacity of the eliminating organ to metabolize the drug is large in comparison with the rate of presentation of drug, clearance will approximate the organ's blood flow. In contrast, when the metabolic capability is small in comparison to the rate of drug presentation, clearance will be proportional to the unbound fraction of drug in blood and the drug's intrinsic clearance. Appreciation of these concepts allows understanding of a number of possibly puzzling experimental results. For example, enzyme induction or hepatic disease may change the rate of drug metabolism in an isolated hepatic microsomal enzyme system but not change clearance in the whole animal. For a drug with a high extraction ratio, clearance is limited by blood flow, and changes in intrinsic clearance due to enzyme induction or hepatic disease should have little effect. Similarly, for drugs with high extraction ratios, changes in protein binding due to disease or competitive binding interactions should have little effect on clearance. In contrast, changes in intrinsic clearance and protein binding will affect the clearance of drugs with low intrinsic clearances and, thus, extraction ratios, but changes in blood flow should have little effect (Wilkinson and Shand, 1975).

Renal Clearance. Renal clearance of a drug results in its appearance as such in the urine; changes in the pharmacokinetic properties of drugs due to renal disease also may be explained in terms of clearance concepts. However, the complications that relate to filtration, active secretion, and reabsorption must be considered. The rate of filtration of a drug depends on the volume of fluid that is filtered in the glomerulus and the unbound concentration of drug in plasma, since drug bound to protein is not filtered. The rate of secretion of drug by the kidney will depend on the drug's intrinsic clearance by the transporters involved in active secretion as affected by the drug's binding to plasma proteins, the degree of saturation of these transporters, and the rate of delivery of the drug to the secretory site. In addition, processes involved in drug reabsorption from the tubular fluid must be considered. The influences of changes in protein binding, blood flow, and the number of functional nephrons are analogous to the examples given above for hepatic elimination.

Distribution

Volume of Distribution. Volume is a second fundamental parameter that is useful in considering processes of drug disposition. The volume of distribution (V) relates the amount of drug in the body to the concentration of drug (C) in the blood or plasma, depending upon the fluid measured. This volume does not necessarily refer to an identifiable physiological volume, but merely to the fluid volume that would be required to contain all of the drug in the body at the same concentration as in the blood or plasma:

$$V = \text{amount of drug in body}/C \qquad (1\text{–}9)$$

A drug's volume of distribution, therefore, reflects the extent to which it is present in extravascular tissues. The plasma volume of a typical 70-kg man is 3 liters, blood volume is about 5.5 liters, extracellular fluid volume outside the plasma is 12 liters, and the volume of total body water is approximately 42 liters. However, many drugs exhibit volumes of distribution far in excess of these values. For example, if 500 μg of digoxin were in the body of a 70-kg subject, a plasma concentration of approximately 0.75 ng/ml would be observed. Dividing the amount of drug in the body by the plasma concentration yields a volume of distribution for digoxin of about 650 liters, or a value almost ten times greater than the total body volume of a 70-kg man. In fact, digoxin distributes preferentially to muscle and adipose tissue and to its specific receptors, leaving a very small amount of drug in the plasma. For drugs that are extensively bound to plasma proteins but that are not bound to tissue components, the volume of distribution will approach that of the plasma volume. In contrast, certain drugs have high volumes of distribution even though most of the drug in the

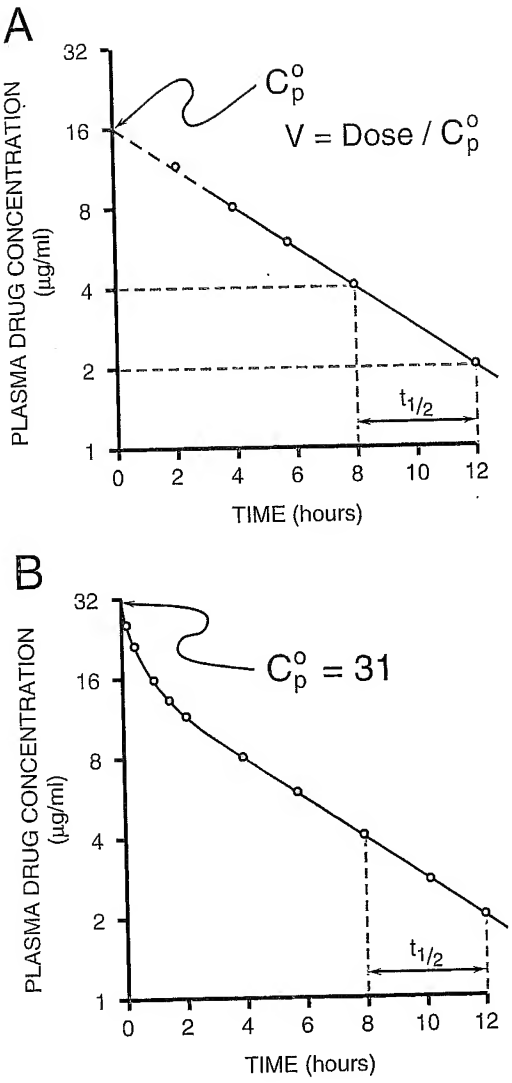


Figure 1–4. Plasma concentration–time curves following intravenous administration of a drug (500 mg) to a 70-kg man.

A. In this example, drug concentrations are measured in plasma from 2 hours after the dose is administered. The semilogarithmic plot of plasma concentration *versus* time appears to indicate that the drug is eliminated from a single compartment by a first-order process [Equation (1–10)] with a half-life of 4 hours ($k = 0.693/t_{1/2} = 0.173\ h^{-1}$). The volume of distribution (V) may be determined from the value of C_p obtained by extrapolation to $t = 0$ ($C_p^o = 16\ \mu g/ml$). Volume of distribution [Equation (1–9)] for the one-compartment model is 31.3 liters or 0.45 liter/kg ($V = dose/C_p^o$). The clearance for this drug is 90 ml/min; for a one-compartment model, $CL = kV$. **B.** Sampling before 2 hours indicates that, in fact, the drug follows multiexponential kinetics. The terminal disposition half-life is 4 hours, clearance is 84 ml/min [Equation (1–5)], V_{area} is 29 liters [Equation (1–11)], and V_{ss} is 26.8 liters. The initial or "central" distribution volume for the drug ($V_1 = dose/C_p^o$) is 16.1 liters. The example chosen indicates that multicompartment kinetics may be overlooked when sampling at early times is neglected. In this particular case, there is only a 10% error in the estimate of clearance when the multicompartment characteristics are ignored. For many drugs multicompartment kinetics may be observed for significant periods of time, and failure to consider the distribution phase can lead to significant errors in estimates of clearance and in predictions of the appropriate dosage. Also, the difference between the "central" distribution volume and other terms reflecting wider distribution is important in deciding a loading dose strategy.

circulation is bound to albumin, because these drugs are also sequestered elsewhere.

The volume of distribution may vary widely depending on the relative degrees of binding to plasma and tissue proteins, the partition coefficient of the drug in fat, and so forth. As might be expected, the volume of distribution for a given drug can differ according to patient's age, gender, body composition, and presence of disease.

Several volume terms commonly are used to describe drug distribution, and they have been derived in a number of ways. The volume of distribution defined in Equation (1–9) considers the body as a single homogeneous compartment. In this *one-compartment model,* all drug administration occurs directly into the central compartment and distribution of drug is instantaneous throughout the volume (V). Clearance of drug from this compartment occurs in a first-order fashion, as defined in Equation (1–3); that is, the amount of drug eliminated per unit of time depends on the amount (concentration) of drug in the body compartment. Figure 1–4*A* and Equation (1–10) describe the decline of plasma concentration with time for a drug introduced into this compartment.

$$C = (dose/V) \cdot exp(-kt) \qquad (1–10)$$

where k is the rate constant for elimination that reflects the fraction of drug removed from the compartment per unit of time. This rate constant is inversely related to the half-life of the drug ($k = 0.693/t_{1/2}$).

The idealized one-compartment model discussed above does not describe the entire time course of the plasma concentration. That is, certain tissue reservoirs can be distinguished from the central compartment, and the drug concentration appears to decay in a manner that can be described by multiple exponential terms (*see* Figure 1–4*B*). Nevertheless, the one-compartment model is sufficient to apply to most clinical situations for most drugs.

Rate of Drug Distribution. The multiple exponential decay observed for a drug that is eliminated from the body with first-order kinetics results from differences in the rates at which the drug equilibrates with tissues. The rate of equilibration will depend upon the ratio of the perfusion of the tissue to the partition of drug into the tissue. In many cases, groups of tissues with similar perfusion/partition ratios all equilibrate at essentially the same rate, such that only one apparent phase of distribution (rapid initial fall of concentration, as in Figure 1–4*B*) is seen. It is as though the drug starts in a "central" volume, which consists of plasma and tissue reservoirs that are in rapid equilibrium with it, and distributes to a "final" volume, at which point concentrations in plasma decrease in a log-linear fashion with a rate constant of k (*see* Figure 1–4*B*).

If the pattern or ratio of blood flows to various tissues changes within an individual or differs among individuals, rates of drug distribution to tissues also will change. However, changes in blood flow also may cause some tissues that were originally in the "central" volume to equilibrate sufficiently more slowly so as to appear only in the "final" volume. This means that central volumes will appear to vary with disease states that cause altered regional blood flow. After an intravenous bolus dose, drug concentrations in plasma may be higher in individuals with poor perfusion (*e.g.*, shock) than they would be if perfusion were better. These higher systemic concentrations may, in turn, cause higher concentrations (and greater effects) in tissues such as brain and heart, whose usually high perfusion has not been reduced by the altered hemodynamic state. Thus, the effect of a drug at various sites of action can be variable, depending on perfusion of these sites.

Multicompartment Volume Terms. Two different terms have been used to describe the volume of distribution for drugs that follow multiple exponential decay. The first, designated V_{area}, is calculated as the ratio of clearance to the rate of decline of concentration during the elimination (final) phase of the logarithmic concentration *versus* time curve:

$$V_{area} = \frac{CL}{k} = \frac{\text{dose}}{k \cdot AUC} \qquad (1\text{–}11)$$

The estimation of this parameter is straightforward, and the volume term may be determined after administration of a single dose of drug by intravenous or oral routes (where the dose used must be corrected for bioavailability). However, another multicompartment volume of distribution may be more useful, especially when the effect of disease states on pharmacokinetics is to be determined. The volume of distribution at steady state (V_{ss}) represents the volume in which a drug would appear to be distributed during steady state if the drug existed throughout that volume at the same concentration as that in the measured fluid (plasma or blood). Following intravenous dosing, estimation of V_{ss} is more complicated than Equation (1–11), but feasible (Benet and Galeazzi, 1979). It is more difficult to estimate V_{ss} following oral dosing. Although V_{area} is a convenient and easily calculated parameter, it varies when the rate constant for drug elimination changes, even when there has been no change in the distribution space. This is because the terminal rate of decline of the concentration of drug in blood or plasma depends not only on clearance but also on the rates of distribution of drug between the "central" and "final" volumes. V_{ss} does not suffer from this disadvantage. When using pharmacokinetics to make drug dosing decisions, the differences between V_{area} and V_{ss} usually are not clinically significant. Nonetheless, both are quoted in the table of pharmacokinetic data in Appendix II, depending upon availability in the published literature.

Half-Life

The half-life ($t_{1/2}$) is the time it takes for the plasma concentration or the amount of drug in the body to be reduced by 50%. For the simplest case, the one-compartment model (Figure 1–4*A*), half-life may be determined readily and used to make decisions about drug dosage. However, as indicated in Figure 1–4*B*, drug concentrations in plasma often follow a multiexponential pattern of decline; two or more half-life terms may thus be calculated.

In the past, the half-life that was usually reported corresponded to the terminal log-linear phase of elimination. However, as greater analytical sensitivity has been achieved, the lower concentrations measured appeared to yield longer and longer terminal half-lives. For example, a terminal half-life of 53 hours is observed for gentamicin (*versus* the more clinically relevant 2- to 3-hour value in Appendix II), and biliary cycling is probably responsible for the 120-hour terminal value for indomethacin (as compared with the 2.4-hour half-life listed in Appendix II). The relevance of a particular half-life may be defined in terms of the fraction of the clearance and volume of distribution that is related to each half-life and whether plasma concentrations or amounts of drug in the body are best related to measures of response. The single half-life values given for each drug in Appendix II are chosen to represent the most clinically relevant half-life.

Early studies of pharmacokinetic properties of drugs in disease were compromised by their reliance on half-life as the sole measure of alterations of drug disposition. It is now appreciated that half-life is a derived parameter that changes as a function of both clearance and volume of distribution. A useful approximate relationship between the clinically relevant half-life, clearance, and volume of distribution at steady state is given by:

$$t_{1/2} \cong 0.693 \cdot V_{ss}/CL \qquad (1\text{–}12)$$

Clearance is the measure of the body's ability to eliminate a drug; thus, as clearance decreases, due to a disease process, for example, half-life would be expected to increase. However, this reciprocal relationship is valid only when the disease does not change the volume of distribution. For example, the half-life of diazepam increases with increasing age; however, it is not clearance that changes as a function of age, but the volume of distribution (Klotz

et al., 1975). Similarly, changes in protein binding of the drug may affect its clearance as well as its volume of distribution, leading to unpredictable changes in half-life as a function of disease. The half-life of tolbutamide, for example, decreases in patients with acute viral hepatitis, exactly the opposite from what one might expect. The disease alters the drug's protein binding in both plasma and tissues, causing no change in volume of distribution but an increase in clearance, because higher concentrations of unbound drug are present (Williams *et al.*, 1977).

Although it can be a poor index of drug elimination, half-life does provide a good indication of the time required to reach steady state after a dosage regimen is initiated or changed (*i.e.*, four half-lives to reach approximately 94% of a new steady state), the time for a drug to be removed from the body, and a means to estimate the appropriate dosing interval (*see* below).

Steady State. Equation (1–1) indicates that a steady-state concentration eventually will be achieved when a drug is administered at a constant rate. At this point, drug elimination [the product of clearance and concentration; Equation (1–3)] will equal the rate of drug availability. This concept also extends to intermittent dosage (*e.g.*, 250 mg of drug every 8 hours). During each interdose interval, the concentration of drug rises and falls. At steady state, the entire cycle is repeated identically in each interval. Equation (1–1) still applies for intermittent dosing, but it now describes the average drug concentration (C_{ss}) during an interdose interval. Steady-state dosing is illustrated in Figure 1–5.

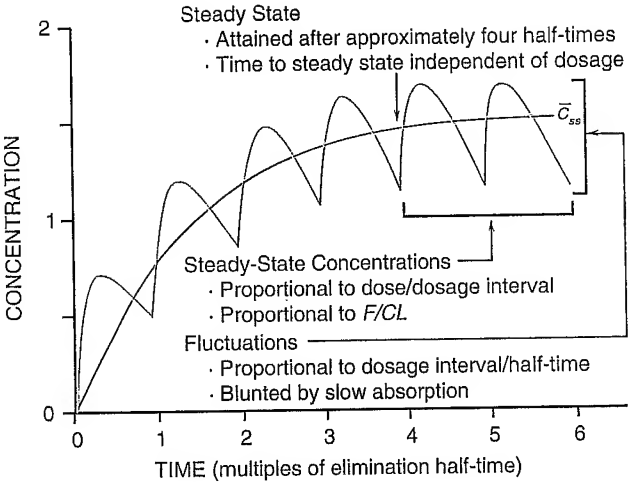


Figure 1–5. Fundamental pharmacokinetic relationships for repeated administration of drugs.

The blue line is the pattern of drug accumulation during repeated administration of a drug at intervals equal to its elimination half-time, when drug absorption is 10 times as rapid as elimination. As the rate of absorption increases, the concentration maxima approach 2 and the minima approach 1 during the steady state. The black line depicts the pattern during administration of equivalent dosage by continuous intravenous infusion. Curves are based upon the one-compartment model.

Average concentration ($\overline{C}_{ss}$) when the steady state is attained during intermittent drug administration:

$$\overline{C}_{ss} = \frac{F \cdot \text{dose}}{CL \cdot T}$$

where F = fractional bioavailability of the dose and T = dosage interval (time). By substitution of infusion rate for $F \cdot \text{dose}/T$, the formula is equivalent to Equation (1–1) and provides the concentration maintained at steady state during continuous intravenous infusion.

Extent and Rate of Bioavailability

Bioavailability. It is important to distinguish between the rate and extent of drug absorption and the amount of drug that ultimately reaches the systemic circulation, as discussed above. The amount of the drug that reaches the systemic circulation depends not only on the administered dose but also on the fraction of the dose, F, which is absorbed and escapes any first-pass elimination. This fraction often is called *bioavailability*. Reasons for incomplete absorption have been discussed above. Also, as noted previously, if the drug is metabolized in the intestinal epithelium or the liver, or excreted in bile, some of the active drug absorbed from the gastrointestinal tract will be eliminated before it can reach the general circulation and be distributed to its sites of action.

Knowing the extraction ratio (E_H) for a drug across the liver [*see* Equation (1–8)], it is possible to predict the maximum oral availability (F_{max}), assuming hepatic elimination follows first-order processes:

$$F_{max} = 1 - E_H = 1 - (CL_{hepatic}/Q_{hepatic}) \qquad (1\text{–}13)$$

Thus, if the hepatic blood clearance for the drug is large relative to hepatic blood flow, the extent of availability will be low when it is given orally (*e.g.*, lidocaine). This reduction in availability is a function of the physiological site from which absorption takes place, and no modification of dosage form will improve the availability under conditions of linear kinetics. Incomplete absorption and/or intestinal metabolism following oral dosing will, in practice, reduce this predicted maximal value of F.

When drugs are administered by a route that is subject to first-pass loss, the equations presented previously that contain the terms *dose* or *dosing rate* [Equations (1–1), (1–5), (1–10), and (1–11)] also must include the

bioavailability term F, such that the available dose or dosing rate is used. For example, Equation (1–1) is modified to:

$$F \cdot \text{dosing rate} = CL \cdot C_{ss} \tag{1–14}$$

Rate of Absorption. Although the rate of drug absorption does not, in general, influence the average steady-state concentration of the drug in plasma, it may still influence drug therapy. If a drug is absorbed rapidly (*e.g.*, a dose given as an intravenous bolus) and has a small "central" volume, the concentration of drug initially will be high. It will then fall as the drug is distributed to its "final" (larger) volume (*see* Figure 1–4*B*). If the same drug is absorbed more slowly (*e.g.*, by slow infusion), it will be distributed while it is being given, and peak concentrations will be lower and will occur later. Controlled-release preparations are designed to provide a slow and sustained rate of absorption in order to produce a less fluctuating plasma concentration–time profile during the dosage interval compared to more immediate-release formulations. A given drug may act to produce both desirable and undesirable effects at several sites in the body, and the rates of distribution of drug to these sites may not be the same. The relative intensities of these different effects of a drug may thus vary transiently when its rate of administration is changed.

Nonlinear Pharmacokinetics

Nonlinearity in pharmacokinetics (*i.e.*, changes in such parameters as clearance, volume of distribution, and half-life as a function of dose or concentration of drug) usually is due to saturation of protein binding, hepatic metabolism, or active renal transport of the drug.

Saturable Protein Binding. As the molar concentration of drug increases, the unbound fraction eventually also must increase (as all binding sites become saturated). This usually occurs only when drug concentrations in plasma are in the range of tens to hundreds of micrograms per milliliter. For a drug that is metabolized by the liver with a low intrinsic clearance/extraction ratio, saturation of plasma-protein binding will cause both V and clearance to increase as drug concentrations increase; half-life may thus remain constant [*see* Equation (1–12)]. For such a drug, C_{ss} will not increase linearly as the rate of drug administration is increased. For drugs that are cleared with high intrinsic clearances/extraction ratios, C_{ss} can remain linearly proportional to the rate of drug administration. In this case, hepatic clearance would not change, and the increase in V would increase the half-time of disappearance by reducing the fraction of the total drug in the body that is delivered to the liver per unit of time. Most drugs fall between these two extremes, and the effects of nonlinear protein binding may be difficult to predict.

Saturable Elimination. In this situation, the Michaelis–Menten equation [Equation (1–2)] usually describes the nonlinearity. All active processes are undoubtedly saturable, but they will appear to be linear if values of drug concentrations encountered in practice are much less than K_m. When they exceed K_m, nonlinear kinetics is observed. The major consequences of saturation of metabolism or transport are the opposite of those for saturation of protein binding. When both conditions are present simultaneously, they may virtually cancel each others' effects, and surprisingly linear kinetics may result; this occurs over a certain range of concentrations for salicylic acid.

Saturable metabolism causes oral first-pass metabolism to be less than expected (higher F), and there is a greater fractional increase in C_{ss} than the corresponding fractional increase in the rate of drug administration. The latter can be seen most easily by substituting Equation (1–2) into Equation (1–1) and solving for the steady-state concentration:

$$C_{ss} = \frac{\text{dosing rate} \cdot K_m}{v_m - \text{dosing rate}} \tag{1–15}$$

As the dosing rate approaches the maximal elimination rate (v_m), the denominator of Equation (1–15) approaches zero and C_{ss} increases disproportionately. Because saturation of metabolism should have no effect on the volume of distribution, clearance and the relative rate of drug elimination decrease as the concentration increases; therefore, the log plasma level-time curve is concave-decreasing until metabolism becomes sufficiently desaturated and first-order elimination is present. Thus, the concept of a constant half-life is not applicable to nonlinear metabolism occurring in the usual range of clinical concentrations. Consequently, changing the dosing rate for a drug with nonlinear metabolism is difficult and unpredictable, since the resulting steady state is reached more slowly, and, importantly, the effect is disproportionate to the alteration in the dosing rate.

Phenytoin provides an example of a drug for which metabolism becomes saturated in the therapeutic range of concentrations (*see* Appendix II). K_m (5 to 10 mg per liter) is typically near the lower end of the therapeutic range (10 to 20 mg per liter). For some individuals, especially children, K_m may be as low as 1 mg per liter. If, for such an individual, the target concentration is 15 mg per liter, and this is attained at a dosing rate of 300 mg per day, then, from Equation (1–15), v_m equals 320 mg per day. For such a patient, a dose 10% less than optimal (*i.e.*, 270 mg per day) will produce a C_{ss} of 5 mg per liter, well below the desired value. In contrast, a dose 10% greater than optimal (330 mg per day) will exceed metabolic capacity (by 10 mg per day) and cause a long and slow but unending climb in concentration until toxicity occurs. Dosage cannot be controlled so precisely (less than 10% error). Therefore, for those patients in whom the target concentration for phenytoin is more than tenfold greater than the K_m, alternating inefficacious therapy and toxicity is almost unavoidable. For a drug like phenytoin that has a narrow therapeutic index and exhibits nonlinear metabolism, therapeutic drug monitoring (*see* below) is most important.

Design and Optimization of Dosage Regimens

Following administration of a dose of drug, its effects usually show a characteristic temporal pattern (Figure 1–6). Onset of the effect is preceded by a lag period, after which the magnitude of the effect increases to a maximum

and then declines; if a further dose is not administered, the effect eventually disappears. This time-course reflects changes in the drug's concentration as determined by the pharmacokinetics of its absorption, distribution, and elimination. Accordingly, the intensity of a drug's effect is related to its concentration above a minimum effective concentration, whereas the duration of this effect is a reflection of the length of time the drug level is above this value. These considerations, in general, apply to both desired and undesired (adverse) drug effects and, as a result, a *therapeutic window* exists reflecting a concentration range that provides efficacy without unacceptable toxicity. Similar considerations apply after multiple dosing associated with long-term therapy; therefore they determine the amount and frequency of drug administration to achieve an optimal therapeutic effect. In general, the lower limit of the therapeutic range appears to be approximately equal to the drug concentration that produces about half of the greatest possible therapeutic effect, and the upper limit of the therapeutic range is such that no more than 5% to 10% of patients will experience a toxic effect. For some drugs, this may mean that the upper limit of the range is no more than twice the lower limit. Of course, these figures can be highly variable, and some patients may benefit greatly from drug concentrations that exceed the therapeutic range, while others may suffer significant toxicity at much lower values.

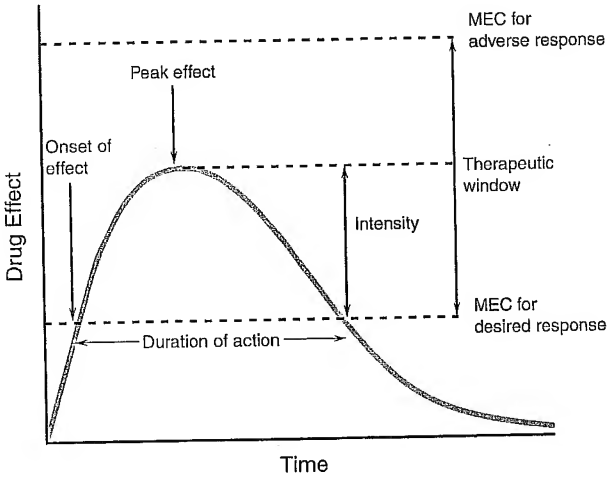


Figure 1–6. Temporal characteristics of drug effect and relationship to the therapeutic window.

A lag period is present before the drug concentration exceeds the minimum effective concentration (MEC) for the desired effect. Following onset of the response, the intensity of the effect increases as the drug continues to be absorbed and distributed. This reaches a peak, after which drug elimination results in a decline in the effect's intensity that disappears when the drug concentration falls back below the MEC. Accordingly, the duration of a drug's action is determined by the time period over which concentrations exceed the MEC. A similar MEC exists for each adverse response, and if drug concentration exceeds this, toxicity will result. Thus, the therapeutic goal should be to obtain and maintain concentrations within the therapeutic window for the desired response with a minimum of toxicity. Drug response below the MEC for the desired effect will be subtherapeutic, whereas above the MEC for an adverse effect, the probability of toxicity will increase. Increasing or decreasing drug dosage shifts the response curve up or down the intensity scale and is used to modulate the drug's effect. Increasing the dose also prolongs a drug's duration of action but at the risk of increasing the likelihood of adverse effects. Accordingly, unless the drug is nontoxic (*e.g.*, penicillins), increasing the dose is not a useful strategy for extending a drug's duration of action. Instead, another dose of drug should be given to maintain concentrations within the therapeutic window.

For a limited number of drugs, some effect of the drug is easily measured (*e.g.*, blood pressure, blood glucose), and this can be used to optimize dosage, using a trial-and-error approach. Even in this ideal case, certain quantitative issues arise, such as how often to change dosage and by how much. These usually can be settled with simple rules of thumb based on the principles discussed (*e.g.*, change dosage by no more than 50% and no more often than every three to four half-lives). Alternatively, some drugs have very little dose-related toxicity, and maximum efficacy is usually desired. For these drugs, doses well in excess of the average required will both ensure efficacy (if this is possible) and prolong drug action. Such a "maximal dose" strategy typically is used for penicillins and most β-adrenergic receptor antagonists.

For many drugs, however, the effects are difficult to measure (or the drug is given for prophylaxis), toxicity and lack of efficacy are both potential dangers, and/or the therapeutic index is narrow. In these circumstances, doses must be titrated carefully, and drug dosage is limited by toxicity rather than efficacy. Thus, the therapeutic goal is to maintain steady-state drug levels within the *therapeutic window.* For most drugs, the actual concentrations associated with this desired range are not and need not be known. It is sufficient to understand that efficacy and toxicity are generally concentration-dependent, and how drug dosage and frequency of administration affect the drug level. However, for a small number of drugs, where there is a small (two- to threefold) difference between concentrations resulting in efficacy and toxicity (*e.g.*, digoxin, theophylline, lidocaine, aminoglycosides, cyclosporine, and anticonvulsants), a plasma-concentration range associated with effective therapy has been defined. In this case, a target level strategy is reasonable, wherein a desired (target) steady-state concentration of the

drug (usually in plasma) associated with efficacy and minimal toxicity is chosen, and a dosage is computed that is expected to achieve this value. Drug concentrations are subsequently measured, and dosage is adjusted if necessary to approximate the target more closely (*see also* Chapter 3).

Maintenance Dose. In most clinical situations, drugs are administered in a series of repetitive doses or as a continuous infusion to maintain a steady-state concentration of drug associated with the therapeutic window. Thus, calculation of the appropriate maintenance dosage is a primary goal. To maintain the chosen steady-state or target concentration, the rate of drug administration is adjusted such that the rate of input equals the rate of loss. This relationship was defined previously in Equations (1–1) and (1–14) and is expressed here in terms of the desired target concentration:

$$\text{Dosing rate} = \text{target } C_p \cdot CL/F \qquad (1\text{–}16)$$

If the clinician chooses the desired concentration of drug in plasma and knows the clearance and bioavailability for that drug in a particular patient, the appropriate dose and dosing interval can be calculated.

Example. Oral digoxin is to be used as a maintenance dose to gradually "digitalize" a 69-kg patient with congestive heart failure. A steady-state plasma concentration of 1.5 ng/ml is selected as an appropriate target. Based on the fact that the patient's creatinine clearance (CL_{CR}) is 100 ml/min, digoxin's clearance may be estimated from data in Appendix II.

$$\begin{aligned} CL &= 0.88\ CL_{CR} + 0.33\ \text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \\ &= 0.88 \times 100/69 + 0.33\ \text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \\ &= 1.6\ \text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \\ &= 110\ \text{ml} \cdot \text{min}^{-1} = 6.6\ \text{liters} \cdot \text{hr}^{-1} \end{aligned}$$

Equation (1–16) is then used to calculate an appropriate dosing rate knowing that the oral bioavailability of digoxin is 70% ($F = 0.7$)

$$\begin{aligned} \text{Dosing rate} &= \text{Target } C_p \cdot CL/F \\ &= 1.5\ \text{ng} \cdot \text{ml}^{-1} \times 1.6/0.7\ \text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \\ &= 3.43\ \text{ng} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \\ &= 236\ \text{ng} \cdot \text{min}^{-1} \text{ for a 69-kg patient} \\ &= 236\ \text{ng} \cdot \text{min}^{-1} \times 60\ \text{min} \times 24\ \text{hr} \\ &= 340\ \mu\text{g} = 0.34\ \text{mg/24 hr} \end{aligned}$$

In practice, the dose rate would be rounded to the closest dosage size, either 0.375 mg/24 hr, which would result in a steady-state plasma concentration of 1.65 ng/ml (1.5 × 375/340), or 0.25 mg/24 hr, which would provide a value of 1.10 ng/ml (1.5 × 250/340).

Dosing Interval for Intermittent Dosage. In general, marked fluctuations in drug concentrations between doses are not desirable. If absorption and distribution were instantaneous, fluctuation of drug concentrations between doses would be governed entirely by the drug's elimination half-life. If the dosing interval (T) was chosen to be equal to the half-life, then the total fluctuation would be twofold; this is often a tolerable variation.

Pharmacodynamic considerations modify this. If a drug is relatively nontoxic, such that a concentration many times that necessary for therapy can be tolerated easily, the maximal dose strategy can be used, and the dosing interval can be much longer than the elimination half-life (for convenience). The half-life of amoxicillin is about 2 hours, but it is often given in large doses every 8 or 12 hours.

For some drugs with a narrow therapeutic range, it may be important to estimate the maximal and minimal concentrations that will occur for a particular dosing interval. The minimal steady-state concentration $C_{ss,\,min}$ may be reasonably determined by the use of Equation (1–17):

$$C_{ss,min} = \frac{F \cdot \text{dose}/V_{ss}}{1 - exp(-kT)} \cdot exp(-kT) \qquad (1\text{–}17)$$

where k equals 0.693 divided by the clinically relevant plasma half-life and T is the dosing interval. The term $exp(-kT)$ is, in fact, the fraction of the last dose (corrected for bioavailability) that remains in the body at the end of a dosing interval.

For drugs that follow multiexponential kinetics and that are administered orally, the estimation of the maximal steady-state concentration $C_{ss,\,max}$ involves a complicated set of exponential constants for distribution and absorption. If these terms are ignored for multiple oral dosing, one may easily predict a maximal steady-state concentration by omitting the $exp(-kT)$ term in the numerator of Equation (1–17) [see Equation (1–18), below]. Because of the approximation, the predicted maximal concentration from Equation (1–18) will be greater than that actually observed.

Example. In the patient with congestive heart failure discussed above, an oral maintenance dosing of 0.375 mg/24 hr of digoxin was calculated to achieve an average plasma concentration of 1.65 ng/ml during the dosage interval. Digoxin has a narrow therapeutic index, and plasma levels between 0.8 and 2.0 ng/ml are usually associated with efficacy and minimal toxicity. It is, therefore, important to know what maximum and minimum plasma concentrations would be predicted with the above regimen. This first requires estimation of digoxin's volume of distribution based on data in Appendix II.

$$\begin{aligned} V_{ss} &= 3.12\ CL_{CR} + 3.84\ \text{liters} \cdot \text{kg}^{-1} \\ &= 3.12 \times (100/69) + 3.84\ \text{liters} \cdot \text{kg}^{-1} \\ &= 8.4\ \text{liters} \cdot \text{kg}^{-1} \\ &= 580\ \text{liters for a 69-kg patient} \end{aligned}$$

Combining this value with that of digoxin's clearance provides an estimate of digoxin's elimination half-life in the patient [Equations (1–1) through (1–12)].

$$\begin{aligned} t_{1/2} &= 0.693\ V_{ss}/CL \\ &= \frac{0.693 \times 580\ \text{liters}}{6.6\ \text{liters} \cdot \text{hr}^{-1}} \\ &= 61\ \text{hr} \end{aligned}$$

Accordingly, the fractional rate constant of elimination is equal to 0.01136 hr^{-1} (0.693/61 hr). Maximum and minimum digoxin plasma concentrations may then be predicted depending upon the dosage interval. If this were every 2 days:

$$C_{ss,max} = \frac{F \cdot \text{dose}/V_{ss}}{1 - exp(-kT)} = \frac{0.7 \times 0.375 \times 2 \text{ mg}/580 \text{ liters}}{0.42} = 2.15 \text{ ng/ml} \quad (1\text{–}18)$$

$$C_{ss,min} = C_{ss,max} \cdot exp(-kT) = (2.15 \text{ ng/ml})(0.58) = 1.25 \text{ ng/ml} \quad (1\text{–}19)$$

Accordingly, the plasma concentrations would fluctuate about twofold, consistent with the similarity of the dosage interval to digoxin's half-life. Also, the peak concentration would be above the upper value of the therapeutic range, exposing the patient to possible adverse effects, and at the end of the dosing interval the concentration would be above but close to the lower limit. By using the same dosing rate but decreasing the frequency of dosing, a much smoother plasma concentration *versus* time profile would be obtained while still maintaining an average steady-state value of 1.65 ng/ml. For example, with a 0.375-mg dose every 24 hours, the predicted maximum and minimum plasma concentrations would be 1.90 and 1.44 ng/ml, respectively, which are in the upper portion of the therapeutic window. By contrast, administering a more conservative dosing rate of 0.25 every 24 hours would produce peak and trough values of 1.26 and 0.96 ng/ml, respectively, that would be associated with a steady-state value of 1.10 ng/ml. Of course the clinician must balance the problem of compliance with regimens that involve frequent dosage against the problem of periods when the patient may be subjected to concentrations of the drug that could be too high or too low.

Loading Dose. The "loading dose" is one or a series of doses that may be given at the onset of therapy with the aim of achieving the target concentration rapidly. The appropriate magnitude for the loading dose is:

$$\text{Loading dose} = \text{target } C_p \cdot V_{ss}/F \quad (1\text{–}20)$$

A loading dose may be desirable if the time required to attain steady state by the administration of drug at a constant rate (four elimination half-lives) is long relative to the temporal demands of the condition being treated. For example, the half-life of lidocaine is usually 1 to 2 hours. Arrhythmias encountered after myocardial infarction obviously may be life threatening, and one cannot wait 4 to 8 hours to achieve a therapeutic concentration of lidocaine by infusion of the drug at the rate required to attain this concentration. Hence, use of a loading dose of lidocaine in the coronary care unit is standard.

The use of a loading dose also has significant disadvantages. First, the particularly sensitive individual may be exposed abruptly to a toxic concentration of a drug. Moreover, if the drug involved has a long half-life, it will take a long time for the concentration to fall if the level achieved was excessive. Loading doses tend to be large, and they are often given parenterally and rapidly; this can be particularly dangerous if toxic effects occur as a result of actions of the drug at sites that are in rapid equilibrium with plasma. This occurs because the loading dose calculated on the basis of V_{ss} subsequent to drug distribution is initially constrained within the initial and smaller "central" volume of distribution. It is, therefore, usually advisable to divide the loading dose into a number of smaller fractional doses that are administered over a period of time. Alternatively, the loading dose should be administered as a continuous intravenous infusion over a period of time. Ideally this should be given in an exponentially decreasing fashion to mirror the concomitant accumulation of the maintenance dose of the drug, and this is now technically feasible using computerized infusion pumps.

Example. "Digitalization," in the patient described above, is gradual if only a maintenance dose is administered (for at least 10 days based on a half-life of 61 hours). A more rapid response could be obtained (if deemed necessary by the physician; *see* Chapter 34) by using a loading dose strategy and Equation (1–20):

$$\text{Loading dose} = 1.5 \text{ ng} \cdot \text{ml}^{-1} \times 580 \text{ liters}/0.7 = 1243 \ \mu\text{g} \sim 1 \text{ mg}$$

To avoid toxicity, this oral loading dose, which also could be intravenously administered, would be given as an initial 0.5-mg dose followed by a 0.25-mg dose at 6 to 8 hours later along with careful monitoring of the patient. It also would be prudent to give the final 0.25-mg fractional dose, if necessary, in two 0.125-mg divided doses separated by 6 to 8 hours to avoid overdigitalization, particularly if there were a plan to initiate an oral maintenance dose within 24 hours of beginning digoxin therapy.

Individualizing Dosage. A rational dosage regimen is based on knowledge of F, CL, V_{ss}, and $t_{1/2}$, and some information about rates of absorption and distribution of the drug. Recommended dosage regimens generally are designed for an "average" patient; usual values for the important determining parameters and appropriate adjustments that may be necessitated by disease or other factors are presented in Appendix II. This "one size fits all" approach, however, overlooks the considerable and unpredictable interpatient variability that usually is present in these pharmacokinetic parameters. For many drugs, one standard deviation in the values observed for F, CL, and V_{ss} is about

20%, 50%, and 30%, respectively. This means that 95% of the time the C_{ss} that is achieved will be between 35% and 270% of the target; this is an unacceptably wide range for a drug with a low therapeutic index. Individualization of the dosage regimen to a particular patient is, therefore, critical for optimal therapy. The pharmacokinetic principles, described above, provide a basis for modifying the dosage regimen to obtain a desired degree of efficacy with a minimum of unacceptable adverse effects. In situations where the drug's plasma concentration can be measured and related to the therapeutic window, additional guidance for dosage modification is obtained. Such measurement and adjustment are appropriate for many drugs with low therapeutic indices (*e.g.*, cardiac glycosides, antiarrhythmic agents, anticonvulsants, theophylline, and others).

Therapeutic Drug Monitoring

The major use of measured concentrations of drugs (at steady state) is to refine the estimate of CL/F for the patient being treated [using Equation (1–14) as rearranged below]:

$$CL/F(\text{patient}) = \text{dosing rate}/C_{ss}(\text{measured}) \qquad (1\text{–}21)$$

The new estimate of CL/F can be used in Equation (1–16) to adjust the maintenance dose to achieve the desired target concentration.

Certain practical details and pitfalls associated with therapeutic drug monitoring should be kept in mind. The first of these relates to the time of sampling for measurement of the drug concentration. If intermittent dosing is used, when during a dosing interval should samples be taken? It is necessary to distinguish between two possible uses of measured drug concentrations to understand the possible answers. A concentration of drug measured in a sample taken at virtually any time during the dosing interval will provide information that may aid in the assessment of drug toxicity. This is one type of therapeutic drug monitoring. It should be stressed, however, that such use of a measured concentration of drug is fraught with difficulties because of interindividual variability in sensitivity to the drug. When there is a question of toxicity, the drug concentration can be no more than just one of many items that serve to interpret the clinical situation.

Changes in the effects of drugs may be delayed relative to changes in plasma concentration because of a slow rate of distribution or pharmacodynamic factors. Concentrations of digoxin, for example, regularly exceed 2 ng/ml (a potentially toxic value) shortly after an oral dose, yet these peak concentrations do not cause toxicity; indeed, they occur well before peak effects. Thus, concentrations of drugs in samples obtained shortly after administration can be uninformative or even misleading.

When concentrations of drugs are used for purposes of adjusting dosage regimens, samples obtained shortly after administration of a dose are almost invariably misleading. The purpose of sampling during supposed steady state is to modify the estimate of CL/F and thus the choice of dosage. Early postabsorptive concentrations do not reflect clearance; they are determined primarily by the rate of absorption, the "central" (rather than the steady-state) volume of distribution, and the rate of distribution, all of which are pharmacokinetic features of virtually no relevance in choosing the long-term maintenance dosage. When the goal of measurement is adjustment of dosage, the sample should be taken well after the previous dose—as a rule of thumb just before the next planned dose, when the concentration is at its minimum. There is an exception to this approach: some drugs are nearly completely eliminated between doses and act only during the initial portion of each dosing interval. If it is questionable whether or not efficacious concentrations of such drugs are being achieved, a sample taken shortly after a dose may be helpful. On the other hand, if a concern is whether or not low clearance (as in renal failure) may cause accumulation of drug, concentrations measured just before the next dose will reveal such accumulation and are considerably more useful for this purpose than is knowledge of the maximal concentration. For such drugs, determination of both maximal and minimal concentrations is thus recommended.

A second important aspect of the timing of sampling is its relationship to the beginning of the maintenance dosage regimen. When constant dosage is given, steady state is reached only after four half-lives have passed. If a sample is obtained too soon after dosage is begun, it will not accurately reflect this state and the drug's clearance. Yet, for toxic drugs, if sampling is delayed until steady state is ensured, the damage may have been done. Some simple guidelines can be offered. When it is important to maintain careful control of concentrations, the first sample should be taken after two half-lives (as calculated and expected for the patient), assuming no loading dose has been given. If the concentration already exceeds 90% of the eventual expected mean steady-state concentration, the dosage rate should be halved, another sample obtained in another two (supposed) half-lives, and the dosage halved again if this sample exceeds the target. If the first concentration is not too high, the initial rate of dosage is continued; even if the concentration is lower than expected, it is usually reasonable to await the attainment of steady state in another two estimated half-lives and then proceed to adjust dosage as described above.

If dosage is intermittent, there is a third concern with the time at which samples are obtained for determination of drug concentrations. If the sample has been obtained just prior to the next dose, as recommended, concentration will be a minimal value, not the mean. However, as discussed above, the estimated mean concentration may be calculated by using Equation (1–14).

If a drug follows first-order kinetics, the average, minimum, and maximum concentrations at steady state are linearly related to dose and dosing rate [*see* Equations (1–14), (1–17), and (1–18)]. Therefore, the ratio between the measured and the desired concentrations can be used to adjust the dose, consistent with available dosage sizes:

$$\frac{C_{ss}\,(\text{measured})}{C_{ss}\,(\text{desired})} = \frac{\text{dose (previous)}}{\text{dose (new)}} \qquad (1\text{–}22)$$

In the previously described patient given 0.375 mg digoxin every 24 hours, for example, if the measured steady-state concentration was found to be 1.65 ng/ml rather than a desired level

of 1.3 ng/ml, an appropriate, practical change in the dosage regimen would be to reduce the daily dose to 0.25 mg digoxin.

$$\text{Dose (new)} = \frac{C_{ss}\text{ (desired)}}{C_{ss}\text{ (measured)}} \times \text{dose (previous)}$$

$$= \frac{1.3}{1.65} \times 0.375 = 0.295 \sim 0.25 \text{ mg/24 hr}$$

Compliance

Ultimately, therapeutic success is dependent on the patient's actually taking the drug according to the prescribed dosage regimen—"Drugs don't work if you don't take them." Noncompliance with the dosing schedule is a major and often unappreciated reason for therapeutic failure, especially in the long-term treatment of disease using antihypertensive, antiretroviral, and anticonvulsant agents. When no special efforts are made to address this issue, only about 50% of patients follow the prescribed dosage regimen in a reasonably satisfactory fashion; approximately one-third only partly comply; and about 1 in 6 patients is essentially noncompliant. Missed doses are more common than too many doses. The number of drugs does not appear to be as important as the number of times a day doses must be remembered (Farmer, 1999). Reducing the number of required dosing occasions will improve adherence to a prescribed dosage regimen. Equally important is the need to involve patients in the responsibility for their own health, using a variety of strategies based on improved communication regarding the nature of the disease and the overall therapeutic plan.

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X. Related Proceedings Appendix

There are no related proceedings.